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The influence of *XRCC1* rs1799782 and *ERCC2* rs13181 genetic polymorphisms in treatment response and prognosis in cervical cancer patients

Daniela Barros Branco

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M.ICBAS **2018**

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THE INFLUENCE OF *XRCC1* rs1799782 AND *ERCC2* rs13181 GENETIC POLYMORPHISMS IN TREATMENT RESPONSE AND PROGNOSIS IN CERVICAL CANCER PATIENTS.

Dissertação de candidatura ao grau de Mestre em Oncologia, no ramo de Oncologia Molecular, submetida ao Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto.

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INFORMAÇÃO TÉCNICA

TÍTULO:

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Setembro de 2018

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1ª EDIÇÃO: Setembro de 2018

Agradecimentos

À Coordenadora do Mestrado em Oncologia, a Professora Carmen Jerónimo, pela oportunidade de ingresar neste mestrado e puder alargar os meus conhecimentos na área da oncologia.

Ao meu orientador, Professor Doutor Rui Medeiros, pela oportunidade de integrar no grupo de Oncologia Molecular e Patologia Viral para desenvolver este trabalho de investigação e por toda a disponibilidade, pelos conselhos e motivação dada ao logo de todas as reuniões.

Ao Mestre Augusto Nogueira por toda a disponibilidade, ajuda, compreesão e paciência que teve comigo ao longo de toda a realização deste trabalho. Também pelo incentivo e apoio nos momentos mais difíceis.

A todos os investigadores presentes no grupo de Oncologia Molecular e Patologia Viral por toda a simpatia, conselhos, disponibilidade para ajudar, por terem tornado a minha passagem por este grupo de investigação numa excelente experiência.

À minha família, em especial aos meus pais, pelo apoio, compreensão e incentivo ao longo da realização deste trabalho, sem eles nada disto era possível.

Ao meu namorado por todo o amor, apoio, compreensão e pela ajuda que me deu na elaboração do trabalho.

A todos os meus amigos, àqueles que me acompanharam ao longo desta etapa, por todo o incentivo e ânimo que me deram.

A todos aqueles que de alguma forma contribuíram para o meu sucesso e para a realização deste trabalho, os meus sinceros agradecimentos.

Abreviations List

A

ATM	Ataxia telangiectasia mutated
ATR	ATM and RAD3-related
AP	Apurinic/apyrimidinic site
APE1	Apurinic/apyrimidinic endonuclease 1
Arg	Arginine
A	Adenine

B

BER	Base Excision Repair
Bp	Base pair

C

CIN	Cervical intraepithelial neoplasia
CT	Computerized Tomography
CCRT	Cisplatin-based concurrent chemoradiation
CHO	Chinese hamster ovary
CR	Complete response
CSA	Cockayne Syndrome group A
CSB	Cockayne Syndrome group B
C	Citosina

D

DNA	Deoxyribonucleic Acid
DDR	DNA damage response
DSB	Double-strand breaks
DSF	Disease-free survival

E

ERCC2	Excision repair cross-complementing group 2
EDTA	Ethlenediamine tetraacetic acid

F

FIGO	*Féderation Internationale de Gynécologie et d'Obstétrique*
FDG	Fluorodeoxyglucose
FEN1	Flap endonuclease 1

G

Gln	Glutamine
GGR	Global genome repair
G	Guanine

H

HPV	Human papillomavirus
HIV	Human Immunodeficiency Virus
HR	Homologous Recombination
His	Histamine
HWE	Hardy-Weinberg equlibrium
HWP	Hardy-Weinberg proportions
HR	Hazard Ratio

I

IR	Ionizing radiation

L

LVSI	Lymphovascular space invasion
LN	Lymph node
LP	Long-patch
Lys	Lysine
LNM	Lymph node metastasis

M

MRI	Magnetic Resonance Imaging
MMR	Mismatch Repair
MPG	N-methylpurine DNA glycosylase

N

NER	Nucleotid Excision Repair
NHEJ	Non-homologous end join
NAC	Neoadjuvant chemotherapy
NEIL1	Nei like DNA glycosylase 1
NSCLC	Non-small cell lung cancer

O

OGG1	8-Oxoguanine DNA glycosylase
OS	Overall survival

P

PET	Positron emission tomography
PTM	Post-translational modifications
PCNA	Proliferating cell nuclear antigen
PARP	Poly (ADP-ribose) polymerase
PNK	Polynucleotide kinase
PR	Partial response
PD	Progression of disease
POLβ	Polymerase beta

R

Rb	Retinoblastoma
RT	Radiation Therapy
ROS	Reactive oxygen species
RPA	Replication protein A
RNA	Ribonucleic acid
RFLP´s	Restriction Fragment Length Polymorphisms
RT-PCR	Real-Time polymerase chain reaction
RECIST	Response Evaluation Criteria In Solid Tumors

S

SCC	Squamous cell carcinoma
SSB	Single-strand breaks
SP	Short-patch
SSBR	Single-stranded break repair
SNP	Single Nucleotide Polymorphism
SD	Stable disease

T

Trp	Tryptophan
TCR	Transcription coupled repair
TFIIH	Transcription factor IIH
T	Timine

U

UV	Ultraviolet light
UNG	Uracil DNA glycosylase
UVC	Ultraviolet C radiation

V

VNTR	Variable Number of Tandem Repeats

W

WHO	World Health Organization

X

XRCC1	X-repair cross complementing group 1
XPA	Xerodema Pigmentosum group A
XPB	Xerodema Pigmentosum group B
XPC	Xerodema Pigmentosum group C
XPD	Xerodema Pigmentosum group D
XPF	Xerodema Pigmentosum group F
XPG	Xerodema Pigmentosum group G

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Resumo

A nível mundial, o cancro do colo do útero é o quarto cancro mais comum em mulheres, e o sétimo em geral, com cerca de 528 mil novos casos diagnosticados em 2012. Existe uma estimativa de 266 mil mortes por esta doença no mesmo ano, representado 7,5% de todas as mortes por cancro feminino.

A infeção pelo vírus do papiloma humano (HPV) é considerada o principal fator de risco para o desenvolvimento desta neoplasia. No entanto, a carcinogénese cervical parece ser dependente de uma série de eventos genéticos e epigenéticos celulares incluindo, entre outros, polimorfismos em genes associados à reparação do DNA (Ácido Desoxirribonucleico). Atualmente existem alguns estudos que sugerem que os polimorfismos genéticos nos genes *XRCC1* (*X-repair cross complementing group 1*) e *ERCC2* (*Excision repair cross-complementing group 2*) parecem reduzir a capacidade de reparação do DNA e com potencial utilidade como marcadores moleculares para prever a resposta terapêutica e o prognóstico de doentes com cancro. O gene *XRCC1* é um dos principais genes de reparação do DNA, estando envolvido na via de reparação por excisão de bases (BER), a qual desempenha um papel fundamental na reparação de pequenas lesões do DNA provocadas por danos de oxidação e alquilação. Por sua vez, o gene *ERCC2* participa na via de reparação por excisão de nucleótidos (NER), através da reparação de ligações cruzadas de DNA e danos provocados por radiações ultravioletas e produtos químicos tóxicos.

Este estudo foi desenvolvido com o objetivo de analisar o efeito dos polimorfismos genéticos *XRCC1* rs1799782 e *ERCC2* rs13181 na evolução clínica de doentes com cancro do colo do útero, nomeadamente na eficácia da resposta terapêutica, sobrevivência global e sobrevivência livre de doença.

Neste trabalho, foi realizado um estudo do tipo coorte retrospetivo de base hospitalar incluindo 260 doentes caucasianas com diagnóstico histopatológico de cancro do colo do útero e estádios entre Ib2 e IVa. Todas as doentes foram admitidas e tratadas com quimioradioterapia concomitante à base de cisplatina, no Instituto Português de Oncologia do Porto. A análise dos polimorfismos genéticos foi realizada por discriminação alélica através da técnica PCR em tempo real e a análise estatística dos resultados foi efetuada com o auxílio do programa estatístico SPSS.

Os resultados obtidos demonstraram na nossa população que mulheres com cancro do colo do útero, gânglios linfáticos negativos e portadoras do genótipo CC do polimorfismo *ERCC2* rs13181 apresentam uma sobrevivência global maior do que as doentes portadoras de um alelo A (p=0.044). Além disso, verificou-se que as doentes com doença avançada, nódulos linfáticos negativos e com o genótipo CC têm uma sobrevivência global maior do que mulheres portadoras de um alelo A (p=0.020). Adicionalmente, os resultados demonstraram que as doentes com doença avançada, idade superior a 39 anos e com o genótipo CC têm uma sobrevivência global maior do que mulheres portadoras de um alelo A (p=0.009) e apresentam um menor risco de recidiva (p=0.040). Utilizando a análise de regressão de Cox observamos que as doentes portadoras do em alelo A apresentam um risco de morte 9 vezes superior às mulheres com genótipo CC (p=0.030).
Por fim, observou-se que as doentes com gânglios linfáticos positivos e portadoras de um alelo C para o polimorfismo *ERCC2* rs13181 ou T para o polimorfismo *XRCC1* rs1799782, têm uma sobrevivência global média menor do que as restantes mulheres (p=0.034).

A realização deste estudo pode contribuir para a definição de um perfil farmacogenómico baseado no *background* genético das doentes com cancro do colo do útero, proporcionando-lhes um tratamento mais individualizado e, consequentemente, uma melhor evolução clínica e uma menor toxicidade.

Palavras-chave: Cancro do colo do útero; *XRCC1*, *ERCC2*; Polimorfismos genéticos; Resposta ao tratamento; Sobrevivência global; Sobrevivência livre de doença.

Abstract

Globally, cervical cancer is the fourth most common cancer in women, and the seventh overall, with about 528,000 new cases diagnosed in 2012. There is an estimated 266,000 deaths from this disease in the same year, representing 7.5% of all deaths from female cancer.

Human papillomavirus (HPV) infection is considered the main risk factor for the development of this neoplasm. However, cervical carcinogenesis appears to be dependent on a number of genetic and cellular epigenetic events including, but not limited to, polymorphisms in genes associated with DNA (Deoxyribonucleic Acid) repair. There are currently some studies that suggest that genetic polymorphisms in *XRCC1* (X-repair cross complementing group 1) and *ERCC2* (Excision repair cross-complementing group 2) genes appear to reduce DNA repair ability and potentially useful as molecular markers to predict the therapeutic response and the prognosis of cancer patients. The *XRCC1* gene is one of the major DNA repair genes, being involved in the base excision repair (BER) pathway, which plays a key role in repairing small DNA damage caused by oxidation and alkylation damage. In turn, the *ERCC2* gene participates in the nucleotide excision repair (NER) pathway, through the repair of DNA cross-links and damage caused by ultraviolet radiation and toxic chemicals.

This study was developed with the objective of analyzing the effect of the *XRCC1* rs1799782 and *ERCC2* rs13181 genetic polymorphisms on the clinical evolution of patients with cervical cancer, namely in the efficacy of the therapeutic response, overall survival and disease-free survival.

In this study, hospital-based retrospective cohort study was performed including 260 Caucasian patients with histopathological diagnosis of cervical cancer and stages between Ib2 and IVa. All patients were admitted to and treated with concomitant chemoradiotherapy based on cisplatin at the Portuguese Oncology Institute of Porto. Genetic polymorphisms analysis was performed by allelic discrimination using the real time PCR technique and the statistical analysis of the results was performed with the aid of the SPSS statistical program.

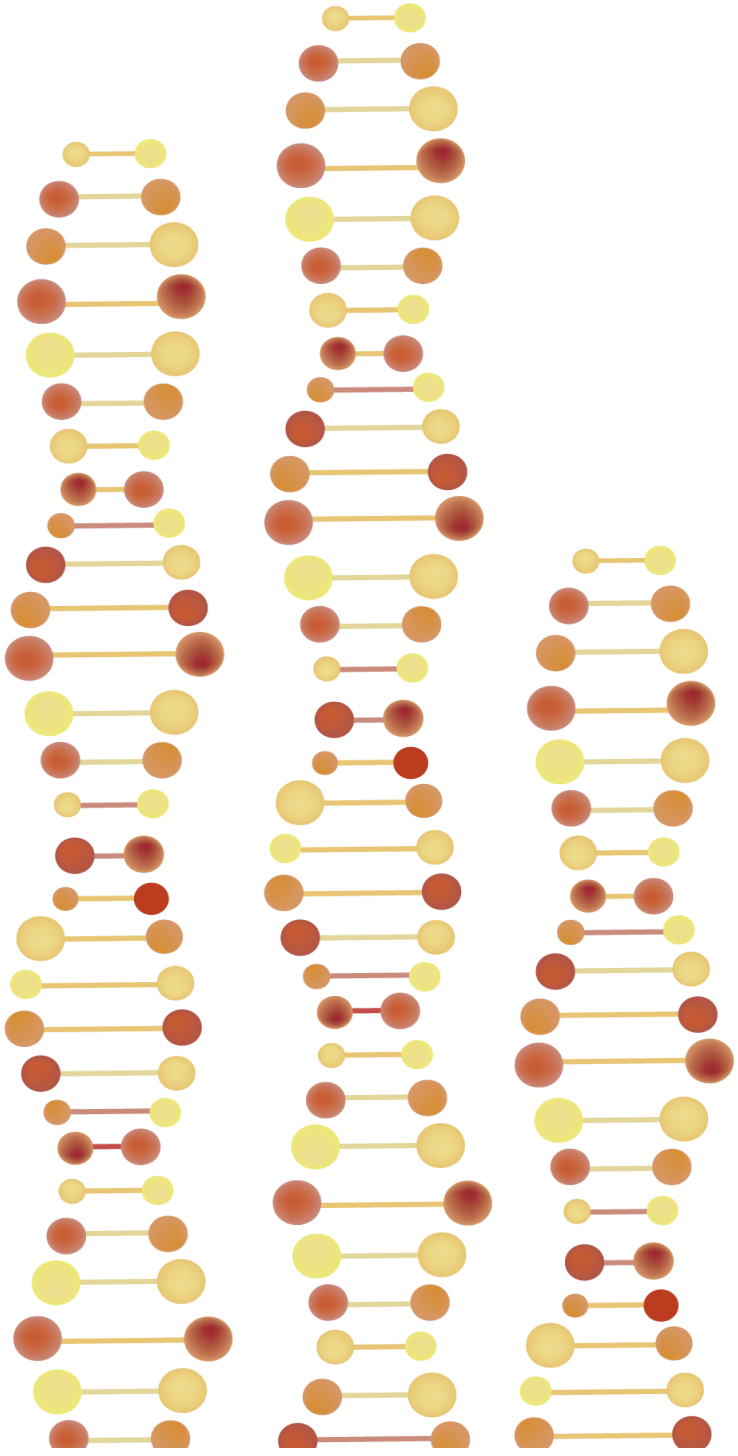
Our results showed that women with cervical cancer, negative lymph nodes and patients with the CC genotype of the *ERCC2* rs13181 polymorphism have an increased overall survival than patients with an A allele (p=0.044). Furthermore, patients with advanced

disease, lymph node negative and CC genotype have been found to have an increased overall survival than women carrier an A allele (p=0.020). Additionaly, among patients with advanced disease and older than 39 years, carriers of the CC genotype have an increased overall survival than women with an A allele (p=0.009) and show a lower risk of relapse (p=0.040). Using Cox regression analysis, we observed that patients with allele A present a 9-fold higher risk of death than women with CC genotype (p=0.030). Finally, patients with lymph nodes positive and carrying a C allele for the *ERCC2* rs13181 or T polymorphism for the *XRCC1* polymorphism rs1799782 were observed to have a decreased overall survival (p=0.034).

The performance of this study may contribute to the definition of a pharmacogenomic profile based on the genetic background of patients with cervical cancer, providing them with a more individualized treatment and, consequently, a better clinical evolution and a lower toxicity.

Key-words: Cervical cancer; *XRCC1*, *ERCC2*; Genetic polymorphisms; Treatment response; Overall Survival; Disease-free survival.

I. INTRODUCTION



1. Cancer: General considerations

Over the years, cancer has been a growing public health problem worldwide, with the incidence rates have increased in most countries since 1990 [1]. This disease is a leading cause of morbidity and mortality worldwide, with about 17 million new cases and more than 8.7 million deaths in 2015, leaving behind only cardiovascular disease. Factors such as aging and population growth are contributing to the increase in the number of new cases, since between 2005 and 2015 it experienced a significant increase of 33%. However, although incidence rates have increased, many countries have suffered a decline in mortality from this disease [2].

In Portugal, 46,724 new cases of cancer were diagnosed in 2010, which corresponded to a cancer incidence rate of 441.9/100,000. The incidence rate was 507.7/100,000 in men (25,658 cases) and 381.7/100,000 in women (21066 cases). There was a 4.5% increase in the number of new cases compared to 2009 [3].

Carcinogenesis is considered multi-stage tumor development process that results from the accumulation of various genetic and epigenetic changes in DNA (Deoxyribonucleic Acid), giving rise to the transformed phenotype and can be divided into three steps: tumor initiation, tumor promotion and tumor progression [4, 5]. The initiation of the tumor consists of the occurrence of changes that cause irreversible genetic damage. Tumor promotion comprises the selective clonal expansion of initiated cells, producing a larger population of cells that are at risk of further genetic changes and malignant conversion [4]. Over of time, many tumors become more aggressive and acquire greater malignant potential. This phenomenon is referred to as tumor progression [5].

As normal cells progressively evolve into a neoplastic state, they acquire a set of hallmarks that allow them to reach a state of malignancy, such as growth factor stimulation, insensitivity to inhibitory signals, activation of invasion and metastization, unlimited replicative potential, induction of angiogenesis and evasion of apoptosis (Figure 1). Underlying all these characteristics, genomic instability, which in turn generates genetic diversity, will speed up the process of acquiring these [6].

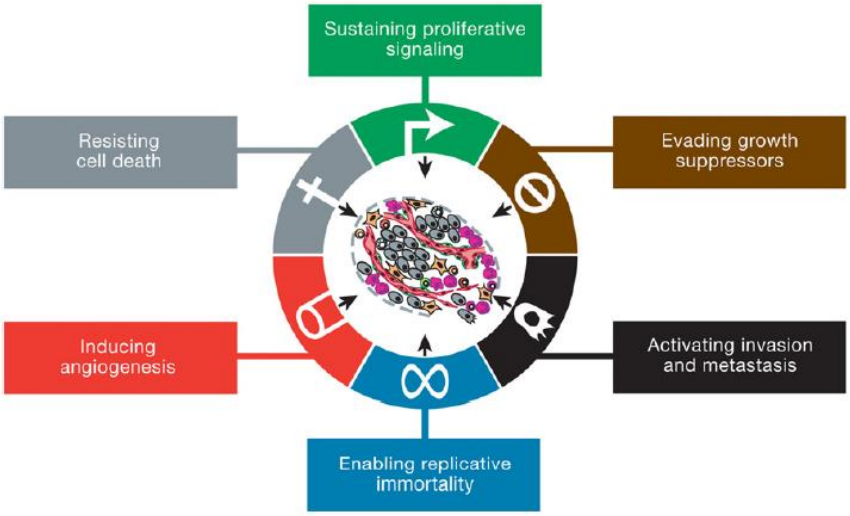


Figure 1 - Hallmarks of cancer (6)

2. Cervical Cancer

2.1. Epidemiology

Cervical cancer is the fourth most common cancer in women, and the seventh overall, with an estimated 528,000 new cases in 2012 and, with a small decrease, 526,000 in 2015 [2, 7]. The discrepancy in cervical cancer incidence and mortality between developed and developing regions has become increasingly apparent, being that 85% of cases and cervical cancer deaths occur in countries with the worst living conditions [8]. There were an estimated 266,000 deaths from cervical cancer worldwide in 2012, accounting for 7.5% of all female cancer deaths, with almost 87% of these deaths occurring in developing countries (Figure 2) [7].

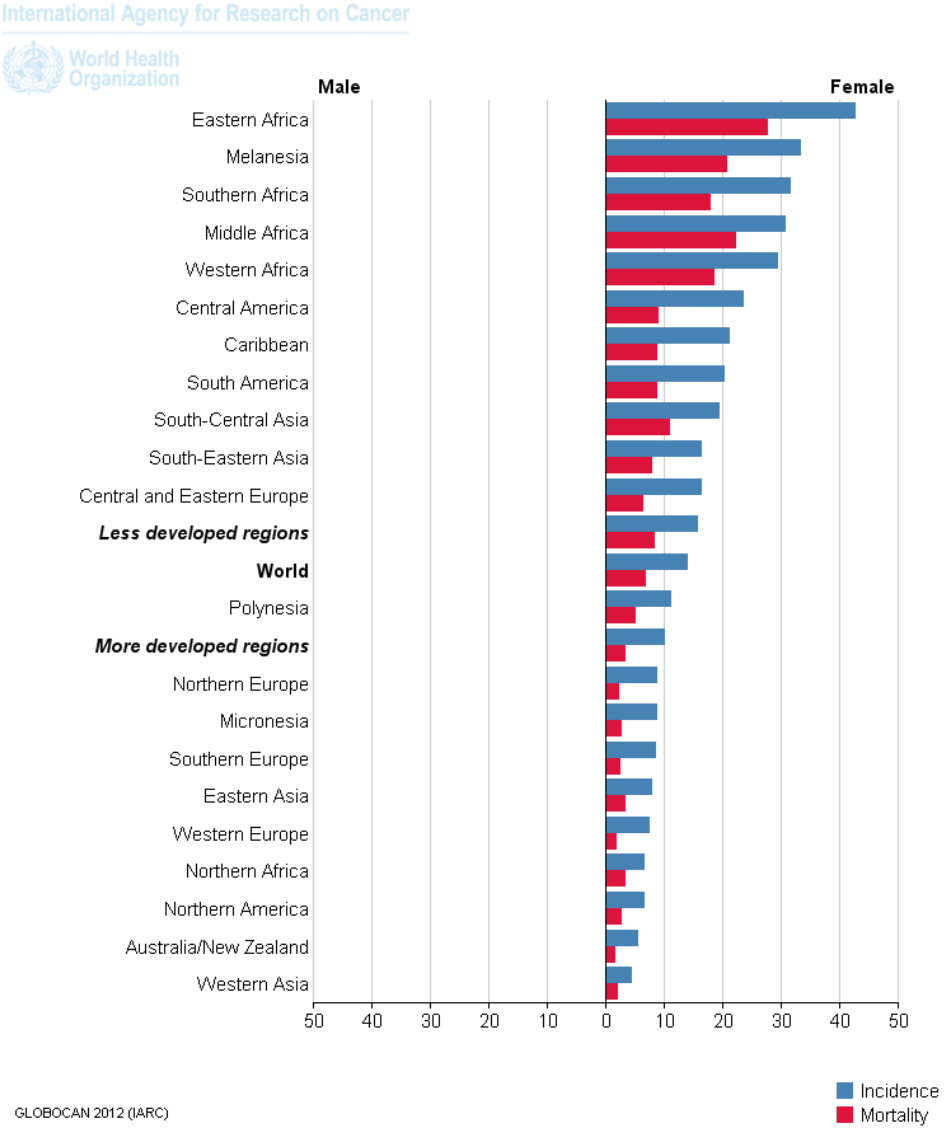


Figure 2 - Estimated age-standardised rates (World) per 100,000 (7)

In Europe, in 2012, an annual incidence was estimated for every 100,000 women of about 13.4 new cases and a mortality of 4.9 deaths. In turn, in Portugal, for the same year was predicted an incidence of 10.8 new cases and a mortality of 4.9 deaths per 100,000 women [9].

Vaccination against Human papillomavirus (HPV) appears to be a promising tool in the primary prevention of cervical cancer, reducing nearly 70% the probability to develop cervical cancer and consequently reducing incidence and mortality rate. Many countries have introduced HPV vaccination into their health-care systems, including Portugal [10].

2.2. Etiology and Risk Factors

The most important risk factor for cervical cancer development is chronic persistent HPV infection [11, 12]. HPVs are a heterogeneous group of double-stranded DNA viruses and infection is a sexually transmitted disease [13]. However, HPV infection alone is an insufficient cause of cervical carcinogenesis. Most HPV infections spontaneously regress and only in a small percentage of cases the infection persists, low-grade intraepithelial lesions progress to highgrade lesions and, ultimately, develop into invasive cervical carcinoma [14].

Several global epidemiological studies indicate that there are 18 different types of high-risk HPVs, namely 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53,56, 58, 59, 66, 68, 73 and 82, which are associated with cervical cancer. HPV16 and HPV18 are the most carcinogenic types within this group, being responsible for approximately 50% and 20% of cervical cancer respectively [15].

Therefore, there are other factors that are associated with increased risk for development of the cervical cancer. As example, women who have many sexual partners or a partner with multiple sex partners are at increased risk for HPV infection and cervical cancer, as well as women who are positive for Human Immunodeficiency Virus (HIV). Long-term use of combined oral contraceptives may also be an important risk factor for this cancer type.

Beside infection with others virus like chlamydia or herpes simplex virus 2 can be associated with chronic inflammation and microulcerative changes of the cervical epithelium that play an important role in initiation and progression of cancer.

General lifestyle factors, including smoking, eating a diet low in fruits and vegetables and being overweight have been considered as co-factors for increase of the risk of cercical cancer. As well as, having a family history of cervical cancer and low socialeconomic conditions or limited access to health care [16].

2.3. Histopathology

Although most infections resolve without consequence, persistent infections can lead to precancerous cervical lesions and, in a minority of women, invasive cancer. Figure 3 shows that the majority of all mild dysplasias regress spontaneously, however a proportion of the high-risk HPV infections will become persistent and, if left untreated, proceed to high-grade lesions and invasive cervical cancer [12]. The most common precancerous lesions are of squamous cell origin, called cervical intraepithelial neoplasia (CIN), and are graded by the proportion of abnormal epithelium (CIN grade 1, 2 and 3). The estimated time for CIN grade 3 progression to cancer is on average 10 years, allowing many opportunities for these lesions to be found and treated [17].

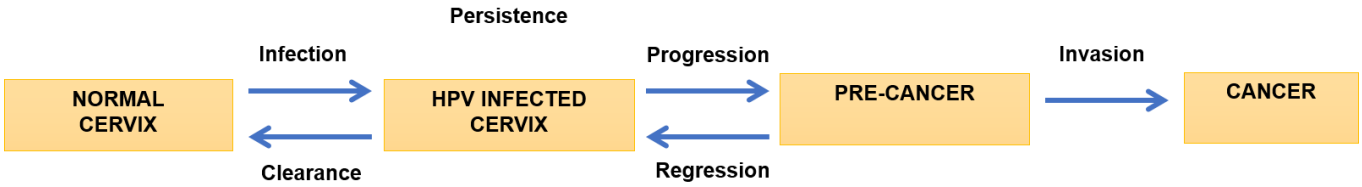


Figure 3 - From HPV infection to cancer: developmental stages of cervical cancer, in which the mild dysplasia regresses spontaneously within less than a year. A proportion of the high-risk HPV (adaptaded 11)

The HPV genome encodes eight proteins, two of which, E6 and E7, account for most of the carcinogenic effects of high-risk types of HPV. These proteins have the ability to promote carcinogenesis by creating genomic instability and by inhibiting tumor suppressor genes. E6 and E7 directly promote genomic instability, which can result in large chromosomal rearrangements and copy number variations, by interfering with centromere duplication during mitosis. Both oncoproteins interfere with important cellular tumor suppressor pathways: E6 inhibits the p53 tumor suppressor by promoting its proteasomal degradation, while E7 disrupts the retinoblastoma (Rb) pathway resulting in uncontrolled activation of the cell cycle and induction of p16INK4A, a cyclin-dependent kinase inhibitor, through a disrupted feedback loop [18].

The World Health Organization (WHO) recognises three categories of epithelial tumours of the cervix: squamous, glandular (adenocarcinoma) and other epithelial tumours including adenosquamous carcinoma, neuroendocrine tumours and undifferentiated carcinoma. Squamous cell carcinomas account for 70%–80% of cervical cancers and adenocarcinomas for 20%–25%.

2.5. Diagnosis, staging and prognostic factors

Cervical tumours are staged using the *Féderation Internationale de Gynécologie et d'Obstétrique* (FIGO) classifications (Table 1). Cervical cancer is the only gynaecological cancer that is clinically staged based on tumour size, vaginal or parametrial involvement, bladder/rectum extension and distant metastases [19]. This staging system is based on physical examination and inspection with limited radiographic evaluation. However nodal status is not included, although is an important determinant in the choice of therapy and prognostic factor [20].

Table 1- The staging of cervical tumours is by the Féderation Internationale de Gynécologie et d'Obstétrique (FIGO) (adaptaded 13)

FIGO stages	Definition
I	Tumour confined to the cérvix*
IA	Invasive carcinoma diagnosed only by microscopy. Stromal invasion with a maximal depth of 5.0mm measured from the base of the epithelium and a horizontal spread of 7.0mm or less**
IA1	Measured stromal invasion 3.0mm or less in depth and 7.0mm or less in horizontal spread
IA2	Measured stromal invasion more than 3.0mm and not more than 5.0mm with a horizontal spread of 7.0mm or less**
IB	Clinically visible lesion confined to the cervix or microscopic lesion greater than T1a/IA2
IB1	Clinically visible lesion 4.0 cm or less in greatest dimension
IB2	Clinically visible lesion more than 4.0 cm in greatest dimension
II	Tumour invades beyond uterus but not to pelvic wall or to lower third of vagina
IIA	Tumour without parametrial invasion
IIA1	Clinically visible lesion 4.0 cm or less in greatest dimension
IIA2	Clinically visible lesion more than 4.0 cm in greatest dimension
IIB	Tumour with parametrial invasion
III	Tumour involves lower third of vagina, or extends to pelvic wall, or causes hydronephrosis or non-functioning kidney
IIIA	Tumour involves lower third of vagina
IIIB	Tumour extends to pelvic wall, or causes hydronephrosis or non-functioning kidney
IVA	Tumour invades mucosa of the bladder or rectum, or extends beyond true pélvis***

*Extension to corpus uteri should be disregarded.

**Vascular space involvement, venous or lymphatic, does not affect classification.

***Bullous oedema is not sufficient to classify a tumour as T4.

Laparotomy, resection of the ovarian mass and hysterectomy are main surgical techniques used for this staging. As well as biopsy of all suspected sites of involvement [21]. Since lymph node metastasis is one of the most importante prognostic factors in patients with early stage cervical cancer, imaging studies also should be done as appropriate [17, 18].

Computerized Tomography (CT) and Magnetic Resonance Imaging (MRI) studies are frequently performed for detection of metastasis in the paraaortic and pelvic lymph nodes, although it is impossible to differentiate metastatic nodes from nonmetastatic hyperplastic nodes of similar size [22]. Furthermore, MRI can determine tumour size, degree of stromal penetrations, parametrial involvement, vaginal extension and corpus extension with high accuracy (19). More recently, it has been shown that positron emission tomography (PET) employing the [18F] -flouro-2-deoxy-D-glucose (FDG) analogue, although more limited in anatomical and spatial resolution, is more sensitive than CT or MRI for detection of lymph node metastases in patients with cervical cancer [23].

Since cervical cancer remains a significant worldwide health challenge and many women die from the disease, it is importante to know de prognostic factors to indicate the best treatment [24]. The most important are lymph node status and number of lymph nodes involved. Tumour size, stage, depth of tumour invasion, lymphovascular space invasion (LVSI) and histological subtype, are other essential factors to take into account [19]. Although the prognostic impact of cervical cancer histology remain inconclusive, some authors have shown that patients with adenocarcinoma have a poorer prognosis than squamous cell carcinoma (SCC) histology [25, 26].

Papanicolaou smear and colposcopy are the main strategies for detecting cervical cancer, but most cases are diagnosed in late stages, especially in developing countries, and are therefore considered a public health problema in this regions [27].

2.6. Treatment and side effects

The treatment decision should be made based on tumor characteristics (size, stage, histology, lymph node (LN) involvement), possible therapeutic complications, requirement for adjuvant therapy and patient choice [28].

Surgery is the mainstay treatment option for early stages of cervical cancer [26, 27]. In the case of microinvasives lesions (FIGO stage IA1 and IA2) that has a low risk of spread beyond the cervix, are usually cured by non-radical operations such as a cone biopsy, trachelectomy (excision of the cervix) or simple hysterectomy [29]. However, some early invasive cancers (stages IA2, IB1 and some small stage IIA tumors) can also be treated with radiotherapy [28].

Standard treatment for locally advanced diseases (stage IIB to IVA) is cisplatin-based concurrent chemoradiation (CCRT) [30, 31]. Cisplatin works synergistically with radiation therapy by preventing the repair of potentially lethal damage induced by radiation,

enhancing sublethal damage, and potentially addressing micrometastatic distant disease [32]. The addition of chemotherapy to radiotherapy (RT) has proved to be more efficient than RT alone in terms of survival benefit, representing a major step forward in optimizing therapy of locally advanced cervical cancer [26, 31]. The standard administration is weekly cisplatin 40mg/m^2 delivered in six weekly treatments, with the last cycle usually coinciding with the final brachytherapy [26, 30].

Although there are many significant advances in combined chemoradiotherapy, distance recurrence continues to be a major cause of treatment failure in patients with invasive cervical cancer. Therefore, a use of new imaging modalities, such as PET-CT, may be importante to guide the choice of therapy [33].

Managing the acute and late toxic effects of treatment is one of the major challenges in cervical cancer therapy [30]. Hematologic toxicity is the most common acute toxicity and include leukopenia, anemia, neutropenia and thrombocytopenia [26, 30, 34]. These effects may limit the number of cycles of chemotherapy received, electrolyte abnormalities, and enteritis [30]. Others possibles acute effects are diarrhea, kidney toxicity and gastrointestinal toxicity [30, 34]. Regarding late complications, gastrointestinal toxicities and genotourinary can affect the quality of life of patients [26, 30].

Radiotherapy also has an effect on healthy cells, and as a consequence there are acute and late side effects that change among patients. Small bowel dysfunction including diarrhea, abdominal cramps, dehydration, electrolyte imbalance and weight loss are some of the early effects of radiotherapy, while vascular changes, fibrosis, stenosis, perforation and necrosis characterize the late effects [35].

2.7. Outcome to treatment

As mentioned, surgery, radiotherapy, chemotherapy and sometimes combination of various therapies are the main therapeutic strategies for cervical cancer. Although these therapies reduce disease symptoms, resistance of cancer cells to chemotherapy agentes and radiotherapy results in the recurrence of tumors [27, 33].

Mechanisms of cellular resistance may include decreased cytotoxic drug accumulation in tumour cells, alterations of detoxification mechanisms, changes in DNA repair enzymes, genomic alterations and regrowth potential [36].

Although resistance of cancer cells to treatment is a primary cause of treatment failure, the causes are frequently multifactorial. Some of these causes may be related with unknown and failure to implement optimal tratment, patient factors, such as multiple pathology or

psychosocial factors. Furthermore, problems of normal tissue tolerance and treatment dose distribution, kinetic factors such as the presence of tumour cells in relatively insensitive phases of the cell cycle and cellular heterogeneity within tumours should also be considered [36].

Response to radiation treatment is known to depend on the well described five R's of radiobiology: damage repair, redistribution in cellular cycle, reoxygenation of cells, cellular repopulation and individual cell radiosensitivity [36]. Moreover, the efficacy of radiotherapy is influenced by physical and biological factors. In relation to biological factors, the most important are the patient’s intrinsic rate of cell proliferation and the extent of hypoxia in the cancer cells. Physical factors include cell kinetics, namely, the rate of proliferation in cancer cells correlates positively with its radiosensitivity and death by irradiation [37].

The efficacy of chemotherapeutics does not only depend on their ability to induce DNA damage, it also depends on the cell's ability to detect and respond to such damage, and the signaling pathways that regulate apoptosis also have a significant impact on the decision of the cellular response to cisplatin [38].

3. DNA repair and cancer

It is estimated that the human genome is constantly exposed to various damaging events per day [39]. The maintenance of genome integrity is important to prevent development of diseases associated with genomic instability including cancer, development genetic defects, infertility, immune deficiency and neurodegenerative disorders [40]. To protect genome integrity after DNA damage, cells active the DNA damage response (DDR), which halts cell cycle progression and facilitates repair of DNA lesions [36, 41].

DDR is a complex signal transduction pathway consisting of a set of strictly regulated steps, the initial detection of DNA damage, the recruitment of DNA repair factors to the site of the lesion and the final repair of the lesions. All components of signaling pathways are functionally categorized into harm sensors, signal transducers and effectors, which are hierarchically organized and communicate with each other [40].

The sensors consist of a group of proteins, that include ataxia telangiectasia mutated (ATM) and ATM-and Rad3-related (ATR) and actively survey the genome for the presence of damage. These proteins signal the damage to three major effector pathways that together determine the result for the cell: checkpoints, DNA repair, and cell death [42, 43]. The whole DDR process is tightly controlled by post-translational modifications (PTM), including

phosphorylation, ubiquitination, sumoylation, methylation, acetylation and others, that been shown to play a pivotal role in this process (Figure 4).

However, it is important to know the DDR defects that are present in a tumor for the selection of optimal treatments because the tumor cells proficient in DDR correct DNA damage induced by the therapy and are more resistant [38, 39].

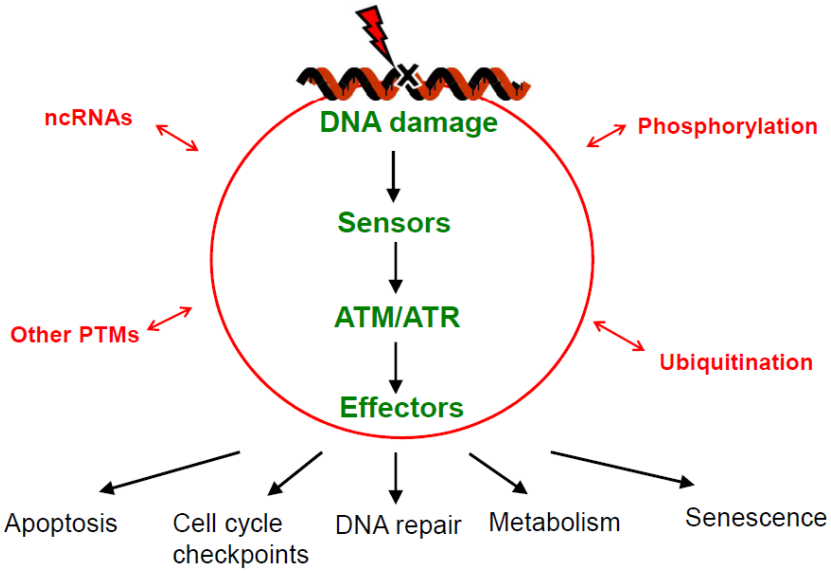


Figure 4 - DNA damage response mechanisms (41)

3.1. Types of damage

Cells are constantly exposed to DNA damaging factors including replication stress, telomere shortening, and a variety of exogenous and endogenous genotoxic insults [44]. Exogenous DNA damages include ultraviolt (UV) light, ionizing radiation (IR) and chronic environmental exposures (eg, cigarettes, asbestos) [40, 41]. However, the vast majority of alterations in DNA are certainly of endogenous origin [45]. This damages comprises numerous chemotherapeutic agents, as well as by-products of normal cell metabolism, namely reactive oxygen species (ROS) [40].

Ionizing radiation induces a variety of DNA lesions, being the most common to double-strand breaks (DSB) [35, 42]. Furthermore, DNA may also indirectly to be damaged by ionizing radiation through the production of ROS [39]. In addition to causing DNA damage, irradiation initiates a signal transduction cascades that maintaining cellular homeostasis and promoting interactions with neighboring cells [46].

Regarding to the direct damage, radiation directly affects the DNA molecules in the target tissue, causing ionization of the direct DNA or holes and electrons transferred to the DNA of its hydration shell [23, 47]. The indirect effect of radiation on DNA molecules includes the formation of free radicals by energy transfer from radiation, resulting in formation of molecular damage caused by the interactions of these free radicals with DNA [28].

Among many chemotherapy drugs that are widely used for cancer treatment, cisplatin is one of the most applied since it has shown effective anticancer activity in a variety of tumors [48]. Cisplatin acts by binding to DNA, leading to DNA adducts formation and consequently intrastrand or interstrand cross-links which disrupt the structure of the DNA molecule, promoting steric changes in the helix, inhibiting DNA replication and drive cells into apoptosis [49].

Regarding to chemoradiotherapy treatment, the use of cisplatin promotes an increase in the number of radiation-induced strand breaks. This my occur due to the conversion of single-strand breaks (SSBs) to double strand breaks during the repair of the platinum adducts. In turn, this conversion or inhibition of repair mechanisms has the effect of increasing the slope of the radiation survival curve and provides a better response to treatment [42].

3.2. DNA repair pathways

To compensate of damage that occur in DNA, cells have developed multiple repair mechanisms specifics for each damage type [39]. Unrepaired damage can result in apoptosis of the cell or can lead to unregulated cell growth and, consequently, cancer developement. Several cellular responses may occur in presence of DNA damages. At cellular level, checkpoints can be activated to arrest cell cycle, transcription can be regulated to compensate for damage, or cell can get in apoptose. Alternatively, DNA damage can be repaired, allowing the cell to reproduce normal [50].

Different DNA repair pathways can be activated to repair different types of DNA lesions that can alter their conformational structure namely, base excision repair, nucleotide excision repair, double strand break repair via homologous recombination (HR) or non-homologous end joining (NHEJ) and mismatch repair (MMR) [51]. BER pathway act in precense of small lesions, such as oxidized or reduced bases, fragmented or non-bulky adducts, or produced by methylating agents, being that the only damaged base is removed by base-specific DNA glycosylases. In contrast, NER pathway repairs bulky lesions such as pyrimidine dimers, larger chemical adducts, and cross-links.

There are at least two DNA double-strand break repair pathways, particulary HR pathway through which DNA ends are resected, posteriorly the exposed 3' single-stranded tails then invade the double helix of the homologous and undamaged partner molecule. Finally, strands are extended by DNA polymerase and cross-overs yield two intact DNA molecules. In its turn, NHEJ repair pathway involves direct ligation of the two damaged double-strand-break ends. Finally, MMR pathway corrects DNA replication errors (base-base or insertion-deletion mismatched) caused by incorrect functioning of DNA polymerase (Figure 5) [50].

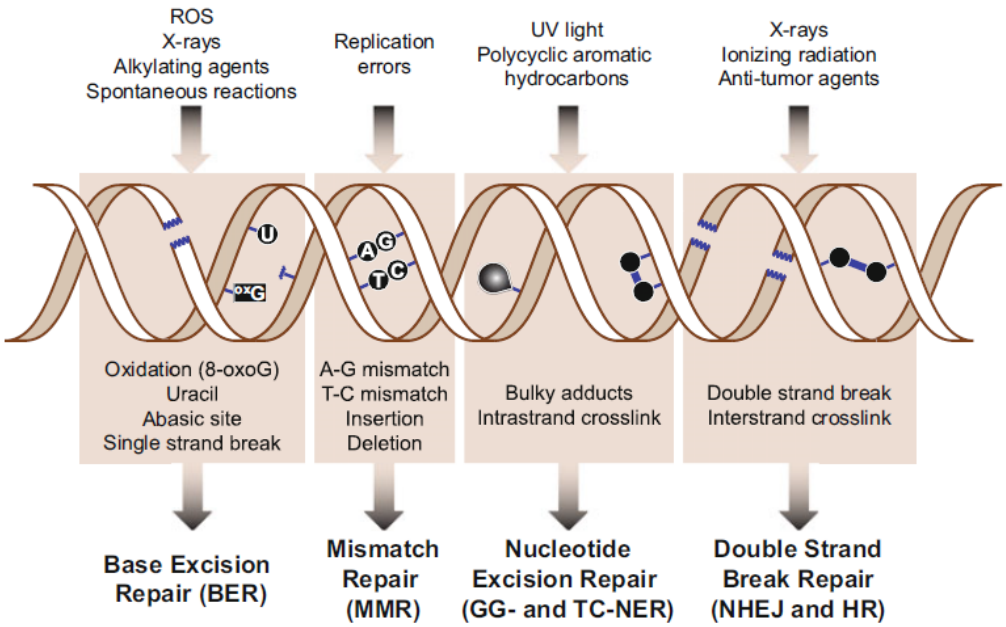


Figure 5 - DNA damage and repair mechanisms (35)

However, the repair of DNA damage can be prejudicial regarding treatment response of cancer cells. So, it is important to take advantage of specific abnormalities in the DNA damage response machinery that are present in cancer cells in order to effectively kill these cells, but not normal cells [52]. For example, changes in the structure of the DNA molecule caused by cisplatin lead to recognition and repair of cellular DNA damage, which can result in continued cell viability, resulting in drug resistance [49]. Thus, based on the principle of synthetic lethality inhibition of the activity of DNA damage response proteins can be used to enhance chemotherapy and radiotherapy efficacy, and also to selectively kill cancer cells showing deficiencies certain DNA repair pathway(s) [52].

Synthetic lethality describes the situation where a defect in one gene or protein is compatible with cell viability but results in cell death when combined (synthesized) with another gene or protein defect [53]. This new therapeutic strategy relies on the frequent defects in the DNA damage response observed in cancer, in which alternative DNA damage response pathways may be activated to allow cancer cells to survive in the presence of

genotoxic stress. Because this strategy targets the cancer-specific aberrations in the DNA damage response, it will cause few or no toxicities on normal cells (Figure 6) [52].

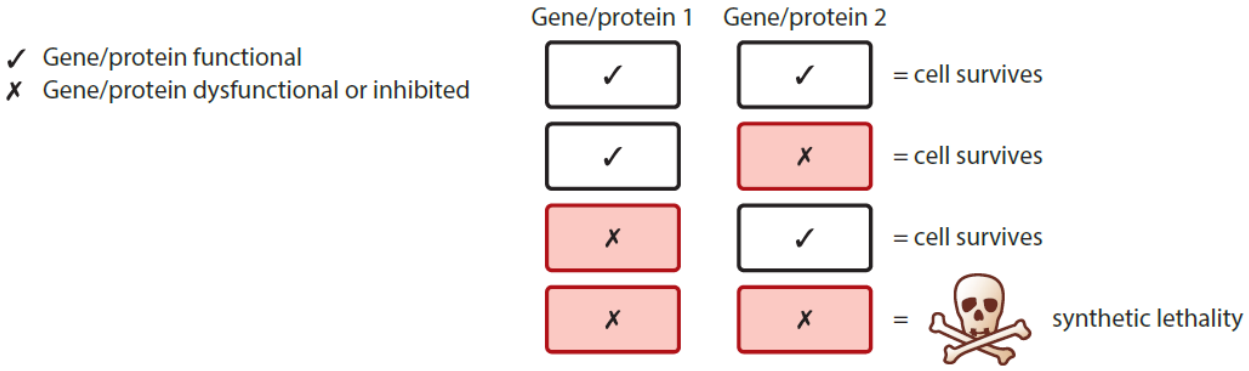


Figure 6 - Scheme of the principle of synthetic lethality (49)

3.2.1. Bases Excision Repair (BER)

Base excision repair is one of the major DNA damage repair pathways. This pathway can be define as a highly coordinated pathway of consecutive enzymatic reactions which deals with the most ubiquitous lesions in DNA, such as oxidative base damage, alkylation, deamination, sites of base loss and single-strand breaks [54].

BER is typically initiated by removal of the damaged base by a DNA glycosylase, resulting in an abasic (AP) site. These AP sites are generally repaired by apurinc/apyrmidinic endonuclease 1 (APE1), that hydrolyzes the phosphodiester backbone immediately 5' to the AP site, creating a single-strand break flanked by 3'-OH and 5'-deoxyribose phosphate (5'-dRP) termini [35, 51, 52]. These primary steps in the BER pathway are common to all organisms.

The synthesis/ligation step is divided into two subpathways, the short-patch (SP) or long-patch (LP) repair. These two alternative routes are distinguished by the size of the repair adhesive: one nucleotide in the case of SP repair and two or more nucleotides in the case of LP repair [55].

These sub-pathways require distinct proteins, the SP repair requires relatively few proteins (DNA glycosylase, APE1, DNA pol β or DNA ligase I or XRCC1/DNA ligase III), whereas the long-patch repair critically depends on flap endonuclease (FEN1) and replicative DNA polymerases δ or ε, both of which require proliferating cell nuclear antigen (PCNA), other replication accessory proteins, and DNA ligase I for optimal activity [55].

Specifically, the sub pathway SP repair, that represents approximately 80-90% of all BER activity, encompasses the filling of a single nucleotide space and the removal of the 5'-dRP reticulum by DNA polymerase β and successive DNA ligation terminates by DNA ligase I or the DNA ligase III complex and XRCC1 protein [39].

The DNA repair by LP-BER subpathway happens in case the AP sites are oxidized or reduced, involving DNA Polδ and Polε. Once the resulting oxidized 5′-dRP cannot be removed by Polβ or by AP lyase-associated DNA glycosylases, FEN1 remove the dRP-containing 5′-termini, leaving gaps spanning several nucleotides. DNA synthesis from these gapped DNA structures was shown to be specifically carried out by Polδ with PCNA as an essential elongation cofactor. Finally, DNA Lig I seals the nicked DNA to complete this BER sub-pathway [56].

XRCC1 plays a crucial role in the coordination of BER's because of its multiple interactions with various repair proteins. Although not catalytically active, XRCC1 is a protein capable of stabilizing and modifying the activity of other proteins. Besides DNA ligase IIIα, XRCC1 interacts with several enzymes involved in the two BER subpathways including APE1 , polymerase beta (Pol β), polynucleotide kinase (PNK), PCNA and poly(ADP-ribose) polymerases 1 and 2 (PARP1/2) [57].

3.2.1.1. XRCC1 gene

XRCC1 gene is a typical DNA repair gene. It is located on chromosome 19 (19q13.2) and consists of 17 exons encoding a 633 amino acid scaffolding protein involved in BER pathway (Figure 7) [58, 59].

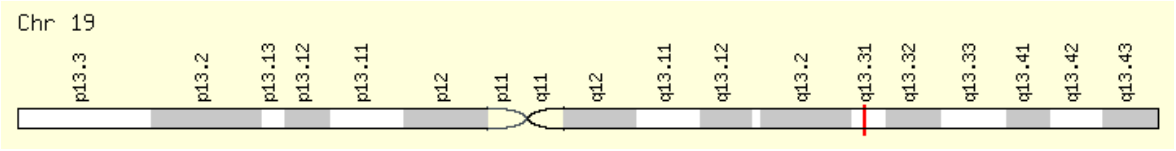


Figure 7 - Localization of the *XRCC1* gene on chromosome 19 (http://www.genecards.org/cgi-bin/carddisp.pl?gene=XRCC1)

The human *XRCC1* gene was identified by complementation of Chinese hamster ovary (CHO) cells that displayed increased sensitivity to X-rays and other DNA damaging agents, particularly those that generate SSBs and base lesions [60]. These cells specifically displayed reduced single-stranded break repair (SSBR) capacity and an increased frequency of sister chromatid exchange. Recently, *XRCC1* deficient cells were found to have reduced initial repair of uracil in DNA as well. Notably, inefficient *XRCC1* associated SSBR is reported to contribute to neurodegenerative disease in humans.

PARPs and XRCC1 proteins are not directly involved in DNA processing, they establish interactions with other BER enzymes for coordinated and efficient reactions. PARP1 possesses an enzymatic activity that polymerizes ADP–ribosyl groups onto many cellular factors including itself. In turn, this recruit XRCC1 which possesses the PAR-binding motif in its central domain and thus interacts with PAR-modified PARP1. Then, PARP1 is dissociated from SSBs because auto-modification results in its decreased affinity for SSBs. XRCC1 then coordinates the BER-repair reactions by interacting with PNKP, Polβ, and LigIIIα, being that the interaction with the last is essential for efficient SSBR. There are other BER proteins that reportedly XRCC1 interacts with to facilitate the whole BER pathway. These include PCNA, APE1, UNG, NEIL1, OGG1, MPG, NTL1, and NEIL2. However, XRCC1 is recruited on SSBs after PARP activation. Although XRCC1 was shown to possess intrinsic affinity for DNA, SSBs are required for efficient interaction of XRCC1 with DNA [56].

However, *XRCC1* participation in DNA repair is not limited to BER/SSBR, *XRCC1* interacting factors have been shown to be involved in NHEJ and to be recruited to UVC induced DNA damage during NER [58].

It were identified nine polymorphisms in *XRCC1* gene, including three commons substitutions: Arg194Trp, Arg280His, and Arg399Gln [61]. Studies have demonstrated that functional single nucleotide polymorphisms (SNPs) of *XRCC1* gene are associated with cancer risks, such as lung cancer, bladder cancer, gastric cancer and other cancers. Furthermore, recente studies suggested that *XRCC1* gene polymorphisms may have a potential role in predicting response to platinum-based chemotherapy in cervical cancer patients [62].

3.2.2. Nucleotide Excision Repair (NER)

Nucleotide Excision Repair is a highly versatile repair pathway that can recognize and remove a wide variety of bulky, helix-distorting lesions from DNA. The most common lesions are bulky covalent adducts, which are formed by nitrogenous bases affected by UV light, ionizing irradiation, electrophilic chemical mutagens, some drugs, and chemically active endogenous metabolites, including reactive oxygen and nitrogen species [63].

This pathway is more complex than BER pathway, requiring the coordinated action of approximately 30 proteins to carry out a multi-step ‘cut-and-patch’-like excision mechanism [35, 63]. These steps envolve recognition ofthe lesion carried out basically by the XPC-hHR23B complex; opening of the double helix at the lesion site by the concerted action of the two DNA helicases XPB and XPD; demarcation of the lesion necessitating the activity

of the XPA and RPA proteins; dual incision of the damaged strand by the XPF and XPG endonucleases; synthesis of DNA in the gap left by the removal of a 24mer–32mer oligonucleotide by the replicative DNA polymerases and PCNA and finally ligation to the parental strand by DNA ligase I [64].

There are two damage recognition and repair sub-pathways of the NER pathway, global genome repair (GGR) and transcription coupled repair (TCR). TCR refers to the preferential repair of transcribed strands in active genes and is is activated by arresting of RNA polymerase II activity at the damaged sites. GGR refers to repair throughout the genome, including that in the non-transcribed strands of active genes and is controlled by XPC, a specialized protein factor that detect the DNA damage [63, 65]. Following damage recognition, both sub-pathways proceed through the common 'core' NER reactions (42).

Initially, either the XPC complex in GG-NER or, CSB and CSA in TC-NER recruit the multi-subunit (ten protein complex) and the multi-functional transcription factor TFIIH to the site of damage. Next, two TFIIH-associated, ATP-dependent helicases XPB and XPD allow the asymmetric unwinding of the DNA helix to form a ~30 nucleotide bubble flanking the lesion: while the XPB protein unwinds the DNA in the 3′ → 5′ direction, the XPD unwinds in the opposite direction [66]. This process permits access of XPA to the damaged region, which provides a second level of damage recognition in addition to ensuring that undamaged DNA is not subjected to excision repair. In the next step, two structure-specific endonucleases XPG and XPF/ERCC1 cleave the DNA at positions 3' and 5' to the damage, respectively, leading to excision of the lesion-containing oligonucleotide of about 30 nucleotides. The NER process is completed by DNA polymerase δ or ε that uses the undamaged strand as a template to resynthesize the resulting gap and lastly, the repaired strand is then sealed by DNA ligase [35, 65, 67].

3.2.2.1. ERCC2 gene

The excision repair cross-complementing rodent repair deficiency group 2 (*ERCC2*) gene, also called the xeroderma pigmentosum group D (*XPD*) gene, is located at chromosome 19q13.3 and comprises 23 exons. The *ERCC2* gene product is a helicase protein of 760 amino acids which belong of the transcription factor IIH (Figure 8) [64].

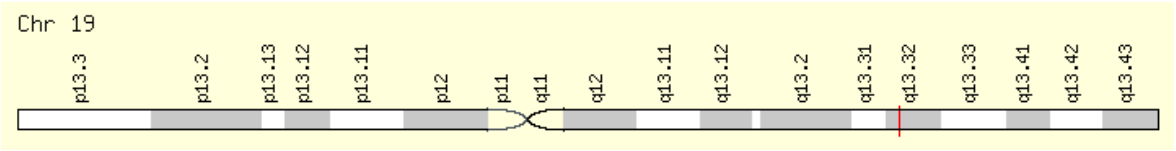


Figure 8 - Localization of the *ERCC2* gene on chromosome 19 (http://www.genecards.org/cgi-bin/carddisp.pl?gene=ERCC2)

ERCC2 protein is a component of the general transcription factor TFIIH complex that plays a key role in nucleotide excision repair (NER) and basal transcription. This protein have a 5'-3' helicase activity and furthermore, plays a bridging function within the TFIIH complex [68].

Point mutations in the human ERCC2 protein play a causative role in DNA repair-deficiency diseases (xeroderma pigmentosum, trichothiodystrophy, and Cockayne syndrome), which are characterized by high ultraviolet-light hypersensitivity, a high mutation frequency, and cancer-proneness, as well as some mental and growth retardation and probably aging [64].

4. Genetic variability and influence in clinical outcome of cancer patients

4.1. Contribution of genetic polymorphisms

Genetic heterogeneity related with neoplasias is important to understand the dynamics of cancer progression and therapeutic resistance [69]. Changes in DNA repair genes can generate genomic instability and contribute to decreased repair capacity, thereby increasing the risk of developing cancer. Thus, it becomes important to study the role of polymorphisms in repair genes in cancer susceptibility and as predictors of response to therapies. Per definition, polymorphisms are heritable variations in the human genome that typically occur in 1% or greater frequency in the population being studied [70-72]. The polymorphisms can be Restriction Fragment Length Polymorphisms (RFLP's), Variable Number of Tandem Repeats (VNTR's) or Single Nucleotide Polymorphisms (SNP's) [73]. Among all kinds of polimorphisms, SNPs are the most abundant, accounting for 90% of known nucleotide variations and consist a base pair (bp) variations at specific locations in the genome. These single nucleotide changes are scattered throughout the genome of all species and forms the basis of human diversity [70, 72-74]. The number of SNPs is estimated to be 19 milion and occur in humans every 300-2000 base pairs (bp) along the genome [69, 70]. SNPs can occur in any region of the genome and depending on where a SNP occurs, it might have different consequences at the phenotypic level (Figure 9). SNPs can occur in noncoding

regions of the genome as well as in genes (introns and exons). SNPs in exonic regions can be of two types: non-synonymous – leading to an amino acid change and affecting the protein – or silent polymorphisms – not leading to an amino acid change and not affecting the protein. SNPs in intronic regions can produce changes in the protein sequence if they are located in a splicing site and can alter gene expression if they are located in a region encoding microRNAs. SNPs in promoter regions can increase, decrease or have no effect in gene expression [74, 75].

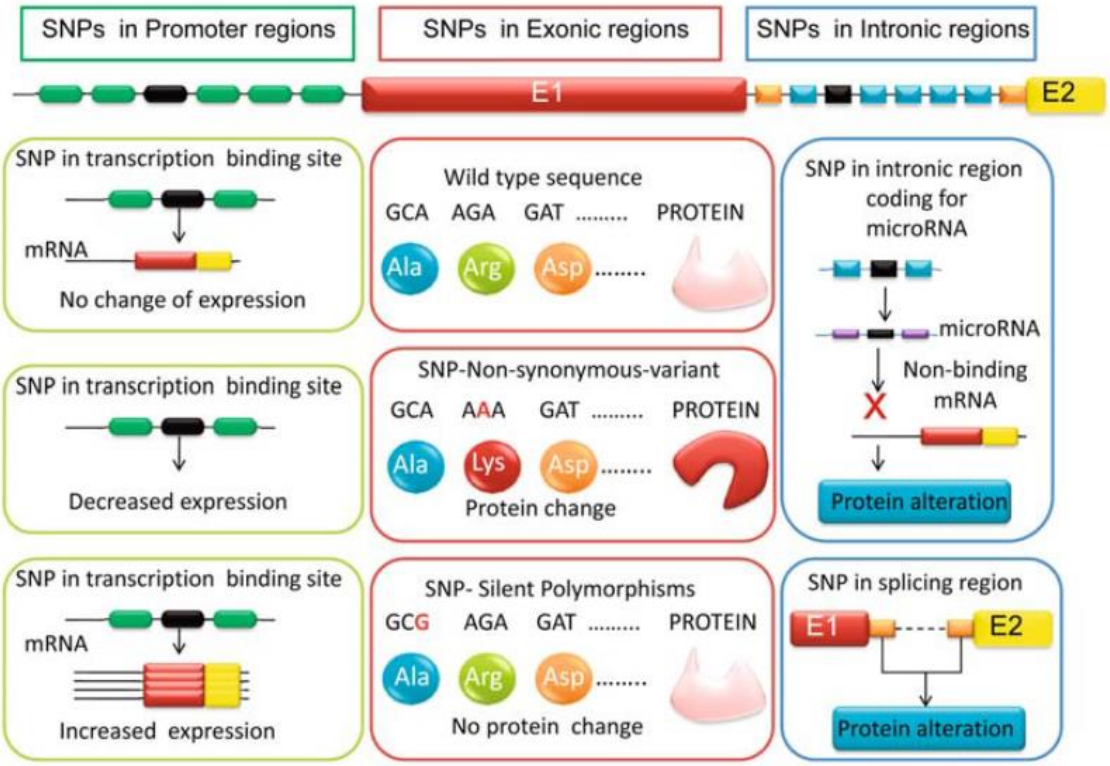


Figure 9 - The location of SNPs and their biological effects (74)

Due to inter-individual differences in DNA damage repair processes, the role of polymorphisms associated with DNA repair genes has been of increasing interest, because they appear to alter the functional properties of DNA repair enzymes and consequently influence the therapeutic response and clinical evolution of cancer patients [50, 76]. It is essential that there is a better understanding regarding the polymorphic variations in the genes and their relationship to disease condition or drug response to be helpful in optimizing personalized therapy and decrease the adverse effects [77].

4.1.1. Genetic polymorphism rs1799782 in the *XRCC1* gene

More than 300 validated SNPs have been identified and described in the *XRCC1* gene, however only three functional SNPs have been extensively studied being that all of which cause amino acid substitutions in the encoded protein [55, 57] [60, 78-81]. One of this three functional polymorphisms in exon 6, consist in the substitution of Cytosine (C) for an Timine (T) at position 26304 of codon 194, which corresponds to an amino acid exchange from a Arginine (Arg) to a Tryptophan (Trp) [81]. As a consequence, substitution of tryptophan with arginine could alter interactions between *XRCC1* gene and POLβ and PARP-1 proteins because the region of exon 6, in which locates Arg194Trp polymorphism encodes a highly conserved hydrophobic linker region between the binding domains of these two proteins [59]. In Europe, the frequency of the C allele is 94,8% and the T allele 5,2%. As for genotype frequencies, these are 90,7%, 1% and 8,3% for genotype CC, TT and CT respectively [82].

The presence of genetic polymorphisms in the *XRCC1* gene have been shown to be associated with cancer susceptibility, including head and neck, esophageal, gastric, breast, lung, colon, cervical cancer and others [59, 76, 80]. Specifically, in relation to the *XRCC1* rs1799782 polymorphism, several studies have been developed to evaluate the risk of carcinogenesis for different types of cancer, including cervical cancer, however the results have been quite inconclusive. Until now, only two tudies evaluate the influence of this polymorphism in the clinical outcome cercical patients [79, 83]. Kim *et al*. showed that the genotypes of *XRCC1* rs1799782 was associated with the therapeutic response [83]. However, another one study did not find any significant association. The inconsistent results may be related with the different ethnic populations and patients number included in studies [79].

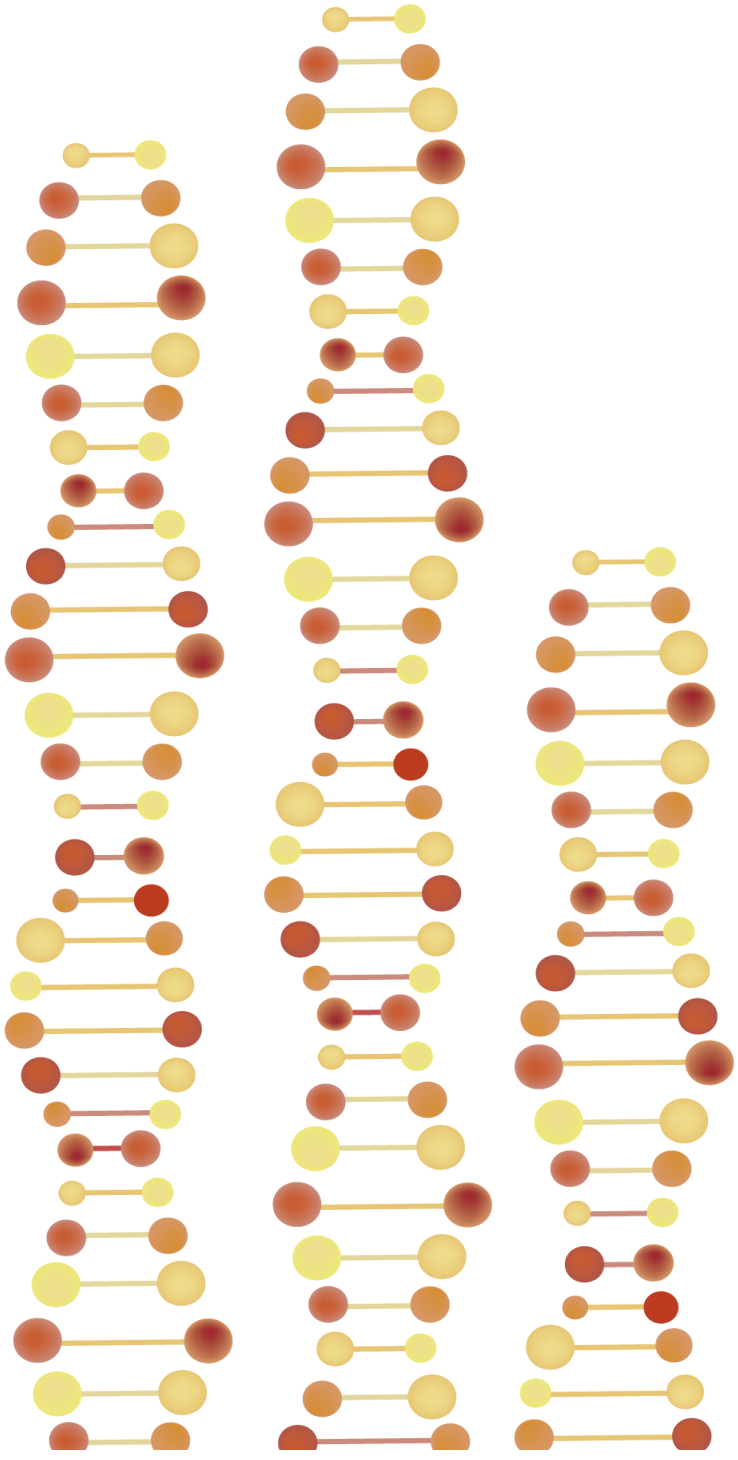
4.1.2. Genetic polymorphism rs13181 in the *ERCC2* gene

Seventeen SNPs in the *ERCC2* gene have recently been detected, of which six are located in exons and eleven in introns. One of six coding region polymorphisms located in exon 23 the substitution of Adenine (A) for a Cytosine (C) at position 35931 of codon 751, which corresponds to an amino acid exchange from a Lysine (Lys) to a Glutamine (Gln). The 751Gln variant completely changes the electronic configuration of the amino acid. This is a major change, located in the important domain of interaction between *ERCC2* protein and its helicase activator, p44 protein, inside the TFIIH complex [62, 82]. This polymorphism was too associated with higher levels of chromatic aberrations and DNA adducts levels [79, 83]. In Europe, the frequency of the A allele is 63,6% and the C allele 36,4%. As for

genotype frequencies, these are 41,7%, 14,5% and 43,7% for genotype AA, CC and AC respectively [84].

Once they are very commons, SNPs in codon 751 is the subject of many epidemiological studies on cancer because may be associated with a reduced repair capacity and increased cancer susceptibility. Due to importance of the polymorphims in codon 751 of *XRCC1* gene, this type genetic variations have been widely studied, namely in various epidemiological studies on cancer because appear to be associated with a reduced DNA repair capacity and, consequently, increased cancer susceptibility [64]. However, regarding the susceptibility of this polymorphism to the development of uterine cervix cancer, no study has yet been performed. Regarding to the influence that this polymorphism may have on the response to treatment and prognosis in patients with cervical cancer, no studies have yet been performed. Therefore, it will be pertinent to conduct this study because of the role of this gene in the DNA repair pathway NER.

II. AIMS



1. General aims

To study the effect of the *XRCC1* rs1799782 e *ERCC2* rs13181 genetic polymorphisms in clinical evolution of patients with cervical cancer treated with concomitant chemoradiotherapy.

2. Specific aims

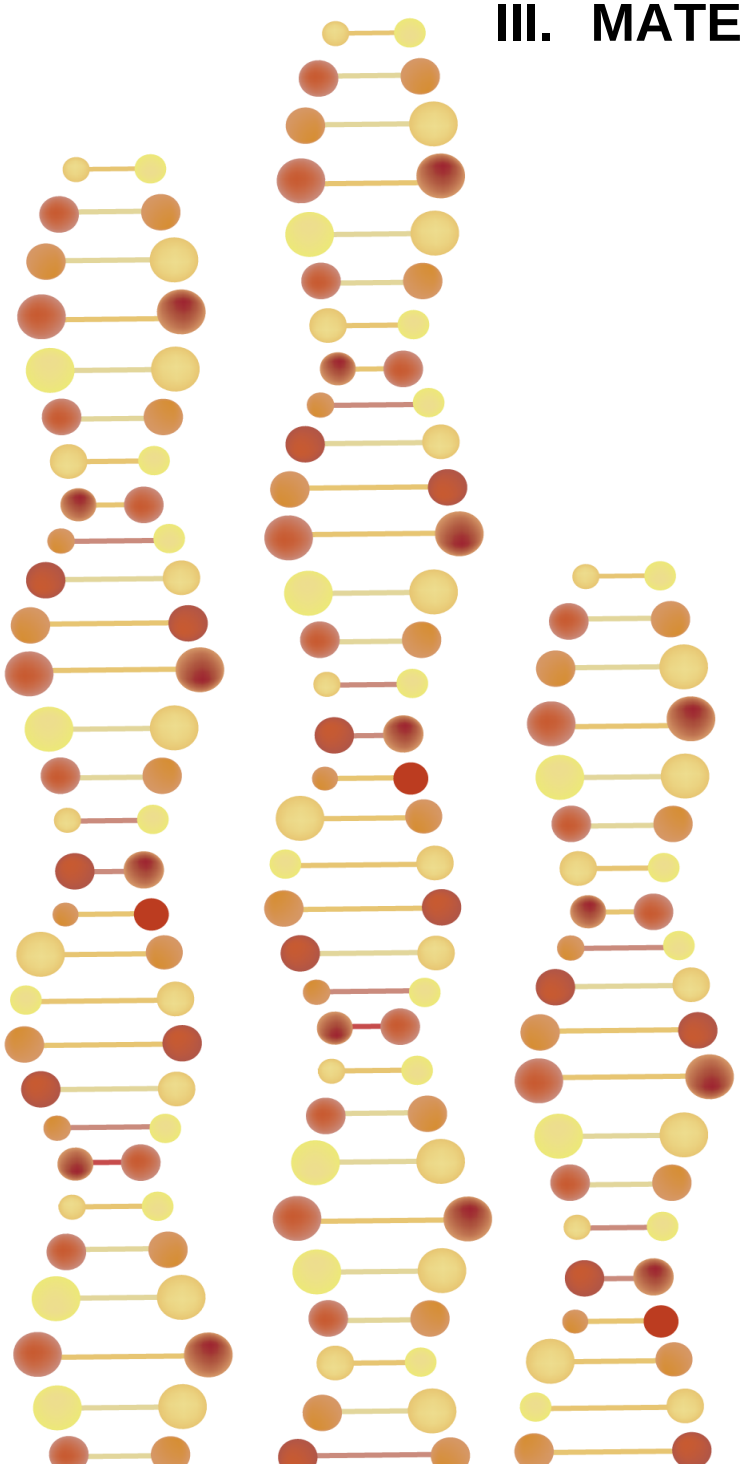
- To optimize the genotyping protocol of the *XRCC1* rs1799782 and *ERCC2* rs13181 genetic polymorhisms.

- To determine the allele and genotype distribution of the polymorphisms selected.

- To compare the allele and genotype frequencies obtained in our study with those observed in other studies from different geographic areas and tumor models.

- To evaluate the possible association between the genetic variants of the *XRCC1* and *ERCC2* genes and therapeutic response, overall survival and disease-free survival in patients with cervical cancer.

III. MATERIALS AND METHODS



1. Characterization of Population

This hospital-based retrospective cohort study was performed including 260 adults patients with histological diagnosis of cervical cancer, at FIGO stages IB2-IVA, recruited between February/2002 to October/2009 from Portuguese Institute of Oncology of Porto. All patients were primarily treated with concurrent chemoradiation and therapeutic protocol consisted in weekly-cisplatin 40 mg/m2 during external radiotherapy. Patients who participated in this study were consecutively selected according to the following inclusion criteria: women with cytologic and histological diagnosis of cervical cancer, age greater than or equal to 18 years, stage Ib2-IVa and treated with concomitant QRT. We excluded patients who underwent surgery before concomitant chemoradiotherapy, cases of lack of informed consent and noncompliance with any of the inclusion criteria.

Peripheral venous blood samples were obtained with the written informed consent of participants prior to their inclusion in the study, according to Helsinki Declaration principles. The study was approved by the ethics committee of Portuguese Institute of Oncology of Porto (CES.287/014).

All data were obtained from medical records and patients' clinical characteristics are described in Table 2.

Table 2 - Patients' clinical characteristics (N = 260)

Clinical characteristic	**n**	**%**
Age, years		
Median, 48.00	260	100
Mean ± SD*, 49.00 ± 11.50		
Histological type		
Squamous cell carcinoma	216	83.1
Adenocarcinoma	32	12.3
Adenosquamous carcinoma	7	2.7
Small cell carcinoma	5	1.9

Tumor stage (FIGO)		
Ib2	22	8.5
IIa2	10	3.8
IIb	163	62.7
IIIa	5	1.9
IIIb	53	20.4
IVa	7	2.7
Nodal invelvement		
Present	14	5.4
No present	246	94.6
Number of chemotherapy cycles		
Median, 6 (range 1 - 6)	260	100
Total dose of radiotherapy		
Median, 80 (range 45 - 88)	260	100
Follow-up time in months		
Median, 63.5 (range 3 - 115)	260	100
Smoking habits		
Smoking	35	13.5
No smoking	152	58.5
No information	73	28.0
Treatment response		
Complete	197	75.8
Partial	45	17.3
Stable	12	4.6
Progression	6	2.3
Recorrence		
Yes	50	19.2
No	210	80.8

*SD= Standard deviation.

2. Laboratory procedures

2.1. Extraction of genomic DNA

For extraction of genomic DNA, about 8mL of peripheral venous blood was collected from all patients through a standard intravenous collection technique for tubes containing ethylenediamine tetraacetic acid (EDTA) for anticoagulant preservation. Genomic DNA was extracted using the GRS Genomic DNA Kit – BroadRange, according to the manufacturer's protocol.

This extraction type of the DNA through system of columns of centrifugation is based on the great affinity of connection of the DNA to the silica membrane that lines the columns of centrifugation. This procedure consists of three phases: adsorption, washing and elution. In first step, for the adsorption to take place, that is, the DNA binding to the silica particles, a buffer solution of high ionic charge and low pH must be used. In addition, ionic resins are present in the alcoholic solution in the membrane, which allows the denaturation of contaminating proteins. Subsequently, several washes are performed with different types of buffer solutions with ethanol, in order to remove proteins, polysaccharides and salts. Finally, DNA is eluted in an elution buffer, of low salt concentration and high pH.

In accordance with manufacturer's instructions, 20µL proteinase K was added to 200µL peripheral blood and the mixture was incubated at 60°C during 5 minutes. Then 200 ml of buffer BR2 was added and mix by shaking vigorously, in order to obtain only the white blood cell fraction. After this procedure, the sample was incubated again at 60°C for 5 minutes. Posteriorly, 200µL of ethanol was added to the lysate, and mix by shaking vigorously immediately for 10 seconds. Subsequently, genomic DNA mini spin column was placed in a 2.0 ml collection tube and sample mixture (including any precipitate if present) was transferred to the column, being posteriorly centrifuged at 14.000g-16.000g for 1 minute. Then biological samples were washed in the column, having been added 400µL of Wash Buffer 1 and centrifuged for 30 seconds. The flow-through was discarted and genomic DNA mini spin column was placed in a new collection tube. Added 600µl of Wash Buffer 2 and centrifuged at 14.000g-16.000g for 30 seconds. The flow-through was discarted and spin column was placed again in a new collection tube and centrifuged for another 3 minutes at 14.000g-16.000g to dry the matrix of the column. In the elution phase, it was necessary transfer the spin column to a new 1,5-ml microcentrifuge tube and pipet 100µl preheated Elution Buffer directly to the centre of the spin column without touching the membrane. After that, the samples were incubated at room temperature for 3-5 minutes. Finally, the samples

were centrifuged for 30 seconds at 14.000g-16.000g to elute purified genomic DNA and stored at -20ºC (Figure 10).

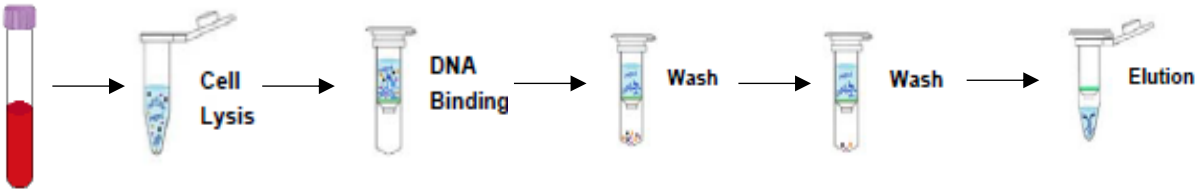


Figure 10 - Scheme of protocol for DNA purification from whole blood

2.2. Genotyping of *XRCC1* rs1799782 and *ERCC2* rs13181 polimorphysms

The *XRCC1* rs1799782 and *ERCC2* rs13181 polymorphisms were selected according to the following criteria: presence of scientific evidence from previous studies in literature; existence of public databases that provided information about the phenotypic risk and biological effect of the polymorphisms and the minor allele had a frequency of at least 10% to 20% in normal population. The characterization of the polymorphisms selected for this study was performed by allelic discrimination using TaqMan methodology (Applied Biosystems) through real-time polymerase chain reaction (RT-PCR) technique. Amplification was detected and analyzed using StepOnePlus™ Real-Time PCR System (Applied Biosystems) software, version 2.3 (Figure 11 and 12).

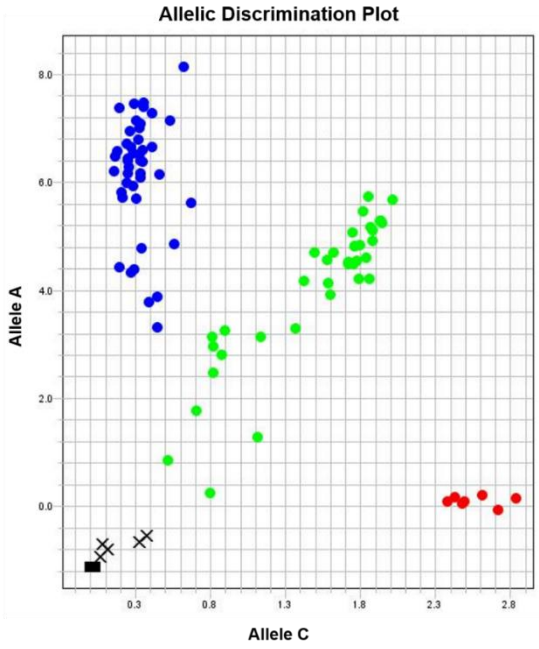


Figure 11 - Example of the representation of a Real Time PCR relative to *ERCC2* rs13181 polymorphism (Blue – AA Homozigous; Green – AC Heterozigous; Red – CC Homozigous; x – Non-amplified cases; Black – Negative controls)

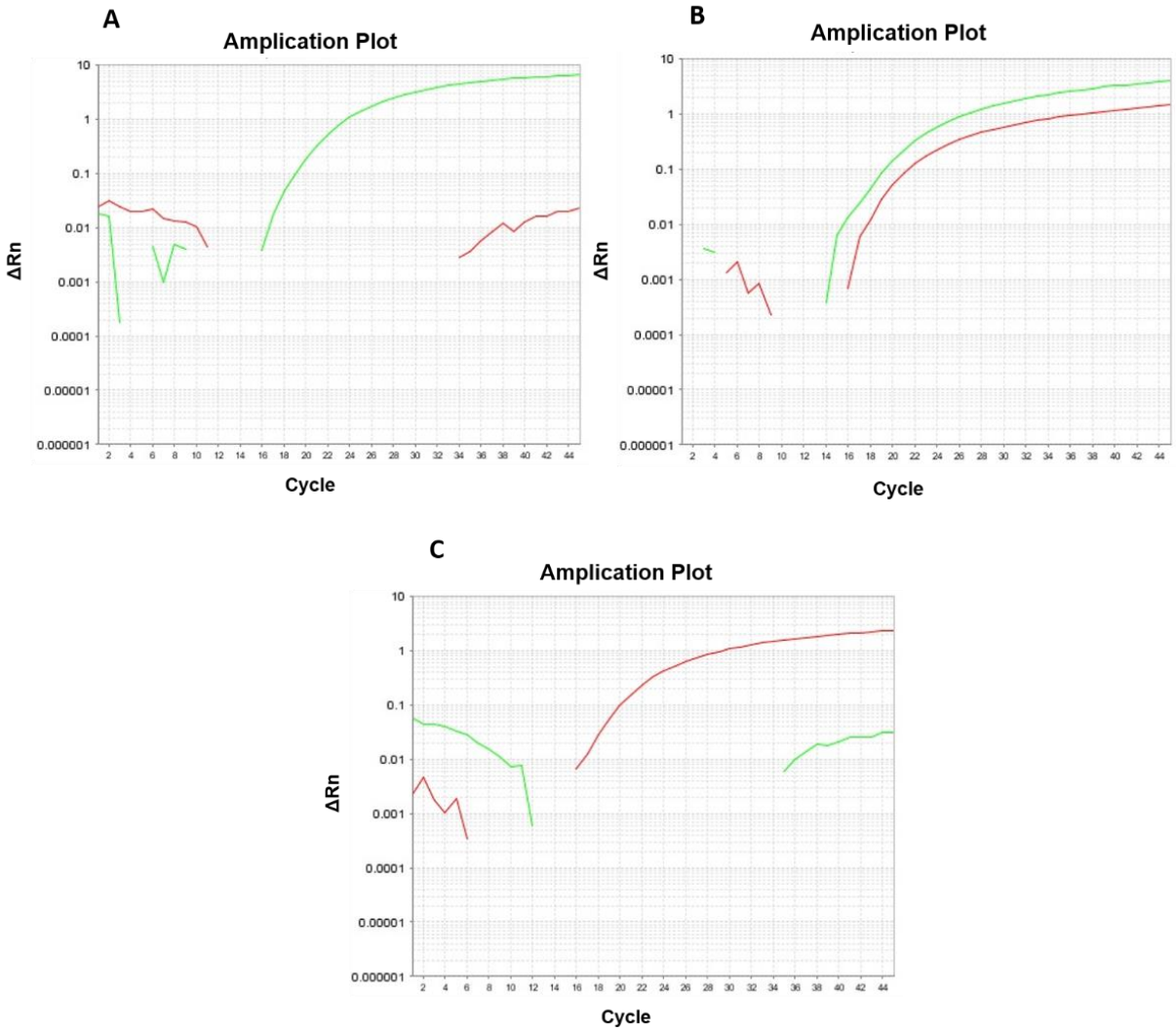


Figure 12 - Representation of fluorescence curves corresponding to each genotype of the *ERCC2* rs13181 polymorphism (A – AA genotype; B – AC genotype; C – CC genotype)

The Real-Time PCR technique has revolutionized the DNA amplification process because it allows the detection and quantification of PCR products in real time as the target DNA is amplified and enables amplification monitoring, increasing its accuracy and reproducibility, and reducing the likelihood of contamination. Allelic discrimination is based in fluorescence emission, through of the use of DNA-bound oligonucleotide probes. Thus, it is necessary that occurs to attach of two fluorometers (VIC® and FAM™) to the 5 'end of each of the allelic discrimination probes, as well as the additional attachment of a minor groove binder (MGB) molecule to 3' end, in order to stabilize the probes in the complementary DNA strand.

These specific probes are normaly labeled by two fluorochromes, a Reporter at the 5' end and a Quencher at the 3' end, which absorbs the fluorescence emited by Reporter fluorochrome, while the probe is intact. Because the 3' end is blocked, these probes can not initiate the synthesis of new DNA strands. During PCR reaction, Taq DNA polymerase synthesizes a new DNA strand, but during amplification of the target sequence, the probe

is cleaved and hydrolyzed by the 5'->3' exonuclease activity of Taq DNA polymerase, leading to release of the Reporter molecule. Thus, as a result of this reaction there is an increase of the fluorescence emission, which will increase exponentially, during each cycle of PCR reaction. The allelic discrimination occurs by selective pairing of one of the probes, in the case of homozygosity or both for heterozygosity, using, for this purpose, the complementary sequence between the forward and reverse primers. The assays used in this study are described in table 3, as well as the respective probes labeled with fluorochromes specific for each allele.

Table 3- Additional information of the assays used and their specific probes

***XRCC1* – rs1799782**	
Assay	C__11463404_10
VIC ®	Allele A
FAM™	Allele G
TCACCTGGGGATGTCTTGTTGATCC**[A/G]**GCTGAAGAAGAGAGCCCCCGGCCTC	

***ERCC2* – rs13181**	
Assay	C___3145033_10
VIC ®	Allele G
FAM™	Allele T
TGCTGAGCAATCTGCTCTATCCTCT**[G/T]**CAGCGTCTCCTCTGATTCTAGCTGC	

For the study of both polymorphisms, the amplification reaction, which completed a final reaction volume of 6µL/case, contained 2.5µL of 2x Taqman Universal Master Mix, 0.125µL of 40x Single Nucleotide Polymorphism Genotyping Assay, 2,375µL of water Braun® sterile double-distilled) and 1µL DNA (~20ng). Amplification conditions were based on the activation of Taq DNA polymerase at 95°C for 10 minutes, followed by 45 cycles of 92°C for 15 seconds for denaturation and 60°C for 1 minute for primer annealing and final extension.

As a quality control in the implementation of the protocol, two negative controls were included in each genotyping reaction to confirm the absence of contamination, genotyping was performed without prior knowledge of the clinical characteristics of the patients and allelic discrimination results were randomly repeated in 10% of samples and analyzed by two independent researchers.

2.3. Evaluation of treatment response

Treatment response was evaluated according to the criteria of Response Evaluation Criteria In Solid Tumors (RECIST). The complete response (CR) indicates the disappearance of the disease, the partial response (PR) designates that at least 50% reduction of tumor extension, stable disease (SD) means that the lesion presents progression less than or equal to 25% or a decrease less than 50%, and progression of disease (PD) indicates a lesion enlargement greater than 25%, or the appearance of a new lesion. In this study, patients with CR were included in the group of good response to therapy and patients with PR, SD and PD classified as having poor response.

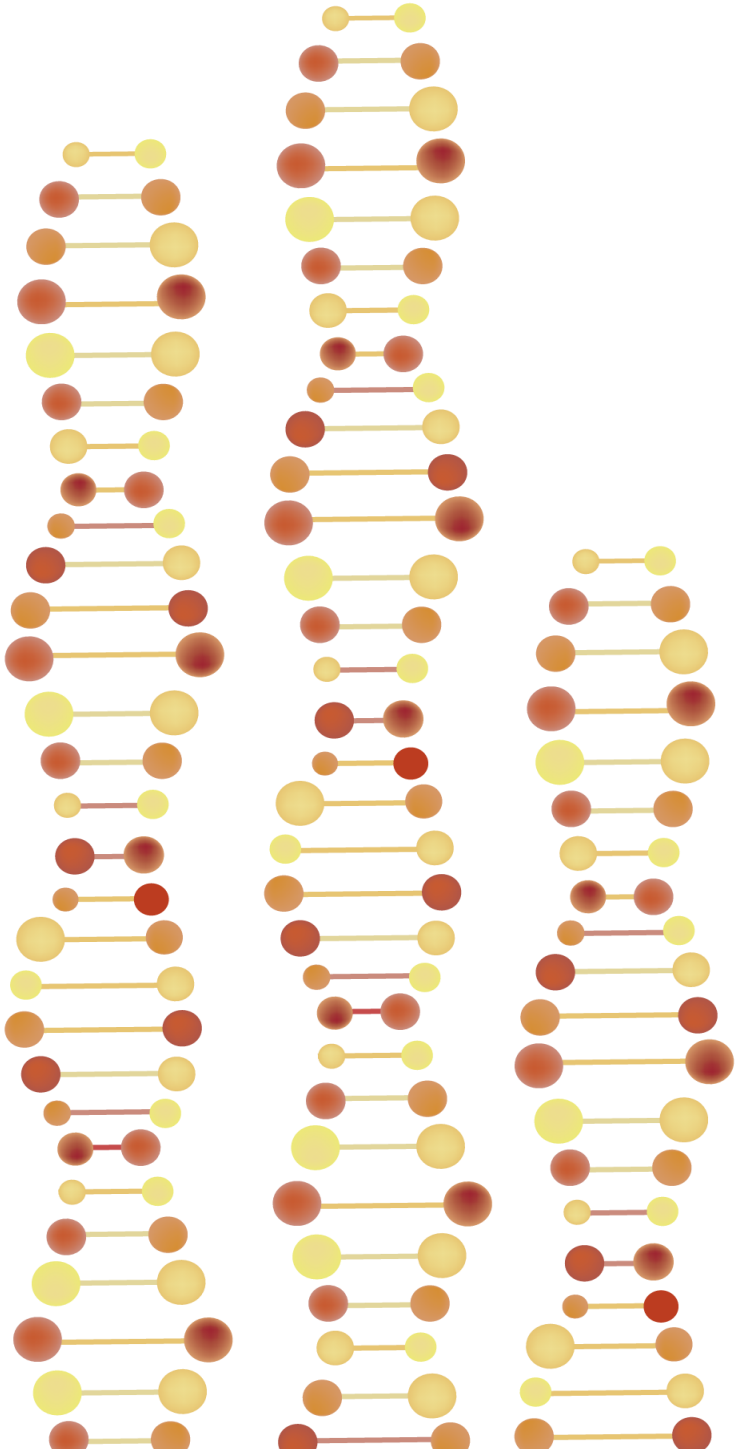
2.4. Statistical analysis

Statistical analysis was performed using SPSS software, version 22.0. The Hardy-Weinberg equilibrium (HWE) was calculated using the goodness-of-fit Pearson test to compare the genotype frequencies observed versus those expected. Analysis by Pearson chi-square (X^2) test was used to compare the different categorical variables, with a significance level of 5%. A p value <0.05 was considered statistically significant.

Overall survival was calculated from the date of diagnosis to the patient's date of death. Dissease free-survival was calculated in patients who achieved complete response to primary treatment, from the date of the end of primary treatment to the date of recurrence, death or last contact (which happened first). Kaplan-Meier method and Log-Rank test were used to obtain and analyze the survival curves.

The relative risk calculation (HR, hazard ratio) of death were estimated by multivariate analysis using Cox regression method. Cox regression model was used to adjust for potential confounders, such as nodal involvement, *ERCC2* rs13181 genotypes fitted as indicator variables.

IV. RESULTS



1. Distribution of genotype and allele frequencies of polymorphisms *XRCC1* rs1799782 and *ERCC2* rs13181

In 260 patients studied, regarding the *XRCC1* rs1799782 polymorphism, genotype frequencies were 87.3%, 12.3% and 0.4% for CC, CT and TT genotypes, respectively. Allele frequencies were 93.5% and 6.5% for the C and T alleles, respectively. As for the *ERCC2* rs13181 polymorphism, 46.9% of the patients are AA genotype, 43.8% AC genotype and 9.2% CC genotype. In this polymorphism, allele frequencies were 68.8% and 31.2%, for the A and C alleles, respectively. The analysis of the Hardy-Weinberg Equilibrium in the study population, according to the genotype frequencies, showed that there are no statistically significant differences between observed and expected frequencies in both polymorphisms (p=0.910 and p=0.721 for *XRCC1* and *ERCC2* polymorphisms, respectively; Table 4).

Table 4- Genotype and allele frequencies of *XRCC1* rs1799782 and *ERCC2* rs13181 polymorphisms in cervical cancer patients

Polymorphism	**Frequencies**		**HWE**
	n	%	*p*
XRCC1			
Genotype			
CC (ArgArg)	227	87.3	
CT (ArgTrp)	32	12.3	0.910
TT (TrpTrp)	1	0.4	
Allele			
C (Arg)		93.5	
T (Trp)		6.5	
Total	**260**	**100**	
ERCC2			
Genotype			
AA (LysLys)	122	46.9	
AC (LysGlu)	114	43.8	0.721
CC (GlnGlu)	24	9.2	
Allele			
A (Glu)		68.8	
C (Lys)		31.2	
Total	**260**	**100**	

HWE – Hardy-Weinberg equilibrium

2. Comparison between allele and genotype frequencies obtained in this study and those observed in other studies of different geographical areas and tumor models

Table 5 shows the allele and genotype frequencies for *XRCC1* rs1799782 and *ERCC2* rs13181 polymorphisms in portuguese population analyzed in this study and in other populations from different geographic areas.

Table 5- Allele and genotype frequencies for the *XRCC1* rs1799782 and *ERCC2* rs13181 polymorphisms in the portuguese population studied and in other populations from different geographic areas

	Population	**N**	**C%**	**T%**	**CC, n (%)**	**CT, n (%)**	**TT, n (%)**	**P**	**Ref.**
	Portuguese	260	93.5	6.5	227 (87.3)	32 (12.3)	1 (0.4)		
XRCC1	European	487	94	6	429 (88)	58 (12)	0	0.385	[85]
	Asian (China)	403	71.6	28.4	202 (50)	173 (43)	28 (7)	<0.001	[86]
	Asian (China)	322	76.4	23.6	204 (63.4)	84 (26.1)	34 (10.5)	<0.001	[87]
	Asian (Thailand)	118	76.7	23.3	65 (55.1)	51 (43.2)	2 (1.7)	<0.001	[88]
	Population	**N**	**A%**	**C%**	**AA, n (%)**	**AC, n (%)**	**CC, n (%)**	**P**	**Ref.**
	Portuguese	260	68.8	31.2	122 (46.9)	114 (43.8)	24 (9.2)		
ERCC2	European	482	66	34	207 (43)	227 (47)	48 (10)	0.582	[85]
		162	62.7	37.3	69 (38.4)	65 (44.3)	28 (17.3)	0.050	[89]
	African	156	75	25	91 (58.3)	52 (33.3)	13 (18.3)	0.072	[90]
	Asian	240	91.7	8.3	201 (83.8)	38 (15.8)	1 (0.4)	<0.001	[91]

By analyzing the results obtained in relation to *XRCC1* rs1799782 polymoprhism, we observed that genotypic distribution in our study was not significantly different from other European population. However, it is possible to verify that there is a significantly difference of the genotypic distribuition between our popupation and Asian population.

For *ERCC2* rs13181 polymorphism, no significantly differences were found in genotype frequencies between our population and European and African populations. However, a statistically significant difference was observed in the distribution of *ERCC2* genotypes between our population and Asian population.

Table 6 shows allele and genotype frequencies for *XRCC1* rs1799782 and *ERCC2* rs13181 polymorphisms observed in other studies involving differents tumor models.

Tabela 6- Allele and genotype frequencies for *XRCC1* rs1799782 and *ERCC2* rs13181 polymorphisms in the portuguese population studied and in other populations with different tumour models

	Cancer	**N**	**C%**	**T%**	**CC, n (%)**	**CT, n (%)**	**TT, n (%)**	**P**	**Ref.**
	Cervical	260	93,5	6,5	227 (87,3)	32 (12,3)	1 (0.4)		
	Cervical	70	65.8	34.2	24 (48.6)	12 (34.3)	36 (17.1)	<0.001	[79]
XRCC1	Cervical	66	68.9	31.1	34 (51.5)	23 (34.9)	9 (13.6)	<0.001	[83]
	Gastric	200	67.5	32.5	85 (42.5)	100 (50.0)	15 (7.5)	<0.001	[92]
	Cancer	**N**	**A%**	**C%**	**AA, n (%)**	**AC, n (%)**	**CC, n (%)**	**P**	**Ref.**
	Cervical	260	68.8	31.2	122 (46.9)	114 (43.8)	24 (9.2)		
	Oral	174	68.1	31.9	79 (45.4)	79 (45,4)	16 (9.2)	0.947	[93]
	Gastrointestinal	80	53.75	46.25	20 (25)	46 (57.5)	14 (17.5)	0.001	[94]
ERCC2	NSCLC	62	0.66	0.34	27 (43.5)	28 (45.2)	7 (11.3)	0.834	[95]
	NSCLC	108	94.4	5.6	96 (88.9)	12 (11.1)	0	<0.001	[96]
	Coloretal	71	58.5	41.5	22 (31.0)	39 (55.0)	10 (14.0)	0.050	[97]

By analyzing the results obtained to *XRCC1* rs1799782 polymorphism, it is possible to affirm that there is a significantly difference of the genotypic distribuition between our popupation and other tumor models (gastric cancer) and other populations with the same tumor model. This may mean that genotypic frequencies of our population with cervical cancer are different to those found in studies including populations with the same or different tumor models.

Regarding the *ERCC2* rs13181 polymorphism, significant differences were found in the genotype frequencies between our population and populations with gastrointestinal and lung cancers. However, no statistically significant difference was observed in the distribution of *ERCC2* genotypes between our population and another population with lung, colorectal and oral cancers. Concerning the differences in the distribution of genotypes between our study and lung cancer populations, these are contradictory, given that exite two studies including lung cancer patients, but with different results.

3. Influence of the polymorphisms *XRCC1* rs1799782 and *ERCC2* rs13181 in treatment response

In order to evaluate the role of the polymorphisms studied in therapeutic response, patients who presented a complete response were considered to be a good response, and patients who presented a partial response, stable disease or progressive disease were considered to be a poor response.

Regarding the response type to treatment, of 260 patients included, 197 presented complete response, 45 partial response, 12 stable disease and 6 progressive disease. Thus, the good response rate was 75.8% and poor response rate was 24.2%. According to the CC, CT and TT genotypes of the *XRCC1* rs1799782 polymorphism, good response rate was 87.8%, 11.7% and 0.5% respectively, and the poor response rate was 85.7%, 14.3% and 0%, respectively. The results showed that there was no significant correlation between the different genotypes and the therapeutic response (p=0.738). As for the *ERCC2* rs13181 polymorphism, the good response rate according to AA, AC and CC genotypes was 47.7%, 43.7% and 8.6%, respectively, and the poor response rate was 44.4%, 44.4% and 11.1%, respectively. For this polymorphism, also no significant association was observed between the genetic variants and the response to treatment (p=0.805) (Table 7).

Table 7- Association of *XRCC1* rs1799782 and *ERCC2* rs13181 polymorphisms with response to chemoradiotherapy in patients with cervical cancer

Polymorphism	**Treatment response**				
	Good response (CR)		Poor response (PR+SD+PD)		
	n	%	n	%	*p*
XRCC1					
CC	173	87.8	54	85.7	
CT	23	11.7	9	14.3	0.738
TT	1	0.5	0	0	
Total	**197**	**100**	**63**	**100**	
ERCC2					
AA	94	47.7	28	44.4	
AC	86	43.7	28	44.4	0.805
CC	17	8.6	7	11.1	
Total	**197**	**100**	**63**	**100**	

CR, complete response; PR, partial response; SD, stable disease; PD, progression of disease

4. Association between overall survival and *XRCC1* rs1799782 and *ERCC2* rs13181 polymorphisms

Evaluation of the influence of polymorphisms under study in overall survival of patients with cervical cancer, it was possible to observe that overall survival time was not statistically different according to *XRCC1* rs1799782 and *ERCC2* rs13181 (p=0.761, Figure 13A and p=0.279, Figure 13B; respectively). In addition, the recessive model for polymorphism of the *ERCC2* gene (CC vs AA and AC) was performed, however there were also no statistically significant differences between genotypes and overall survival (p=0.122, Figure 14). For the *XRCC1* gene polymorphism it was not possible to make the recessive model because there is only one carrier patient of the TT genotype.

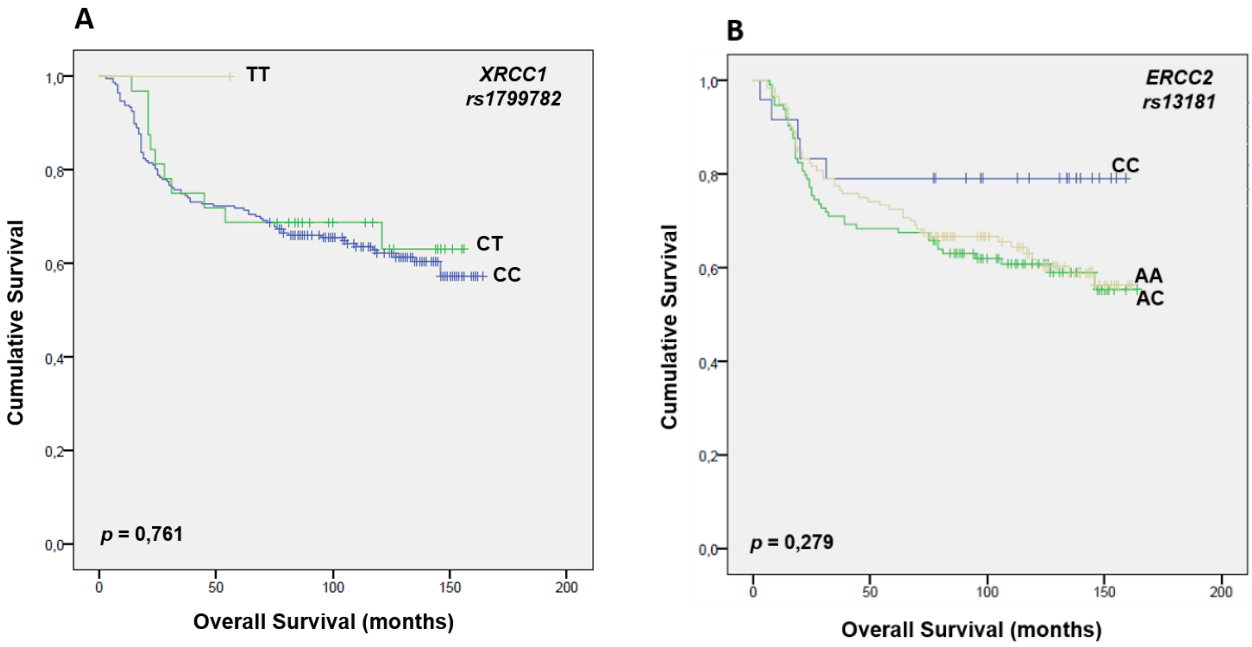


Figure 13 - Overall survival curves obtained by the Kaplan-Meier method and log rank test in cervical cancer patients, according to the genotypes of the *XRCC1* rs1799182 polymorphism (nTT=1; nCT=32; nCC=227; Figure 13A) and *ERCC2* rs13181 polymorphism (nCC=24; nAC=114; nAA=122; Figure 13B)

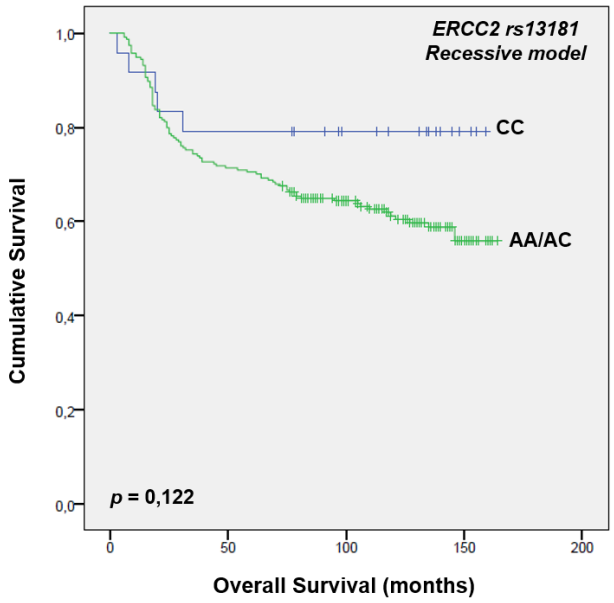


Figure 14 - Overall survival curves obtained by the Kaplan-Meier method and log rank test in cervical cancer patients, according to the recessive model for *ERCC2* rs13181 polymorphism (nCC=24; nAC=114; nAA=122)

Additionally, considering the recessive model for *ERCC2* rs13181 polymorphism, a statistical analysis was performed according to some clinical-pathological data, such as the stage, lymph node metastasis (LNM) and age. Through this analysis, it was found that there is a statistically significant difference in overall survival between patients with negative

lymph nodes and different genotypes. Thus, we observed that patients carrying the CC genotype have a higher mean overall survival than women with at least one A allele (*p*=0.044, Figure 15).

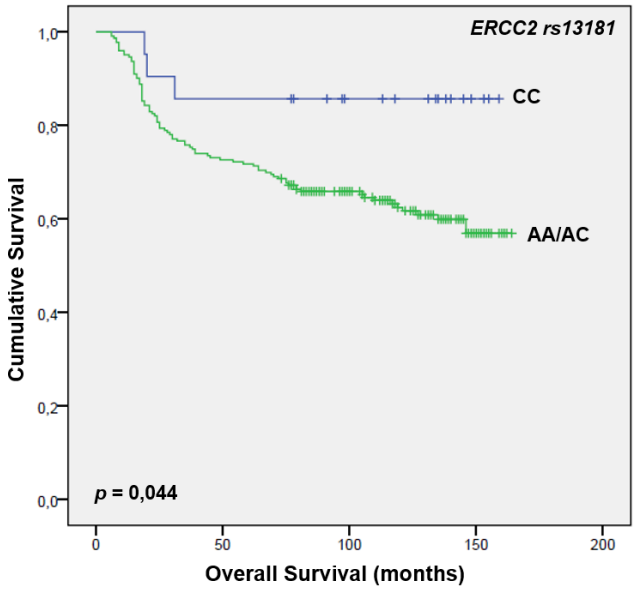


Figure 15 - Overall survival curves obtained by the Kaplan-Meier method and log rank test in cervical cancer patients with negative lymph nodes, according to the recessive model for *ERCC2* rs13181 polymorphism (nCC=21; nAA/AC=223)

Furthermore, it was found that there also exists a statistically significant difference in overall survival between patients with locally advanced disease and negative lymph nodes and different genotypes. So, we observed that patients carrying the CC genotype have a higher mean overall survival time than women with at least one A allele (*p*=0.020, Figure 16).

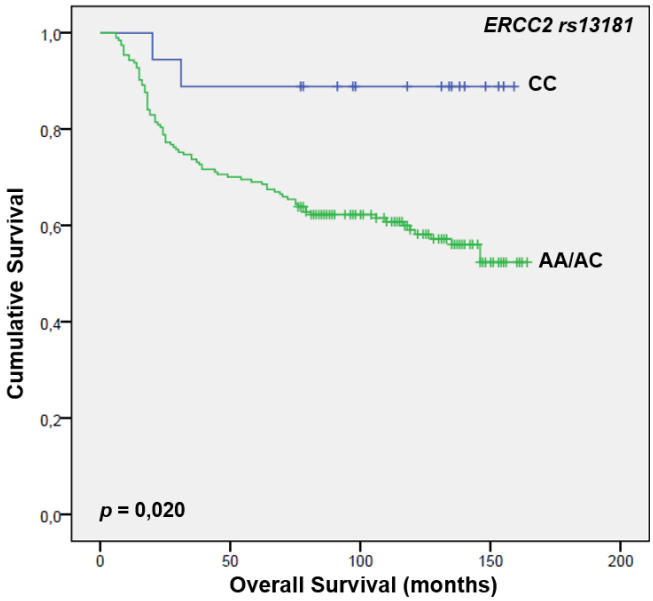


Figure 16 - Overall survival curves obtained by the Kaplan-Meier method and log rank test in cervical cancer patients with locally advanced disease and negative lymph nodes, according to the recessive model for *ERCC2* rs13181 polymorphism (nCC=18; nAA/AC=194)

In this study, the results also showed that there is a statistically significant difference in overall survival between women with locally advanced disease and age greater than 39 years and different genotypes. So, we observed that patients carrying the CC genotype have a higher mean overall survival than women with at least one A allele (p=0.009, Figure 17). Additionally, by multivariate Cox regression model, adjusted for LNM prognostic factor (p=0.128), it was also possible to verify that women carriers of AA/AC genotypes present a risk of death of approximately 9 times higher than women with CC genotype [hazard ratio (HR), 8.92; 95% confidence interval (95% CI), 1.24–64.12; p=0.030; p=0.029, bootstrap analysis].

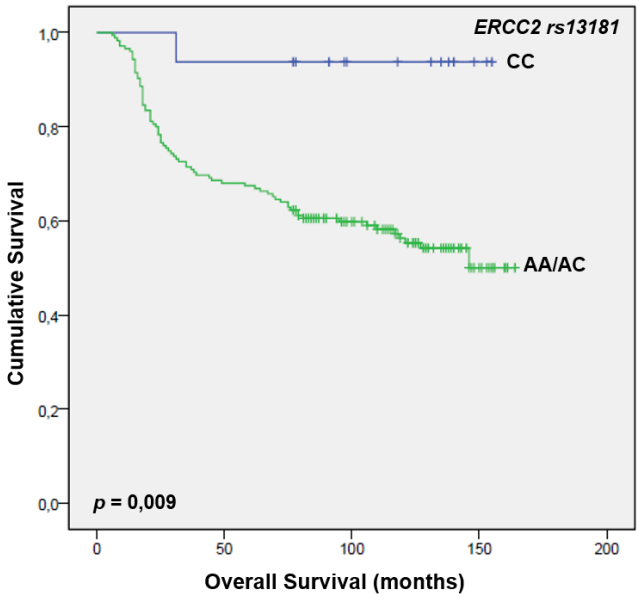


Figure 17 - Overall survival curves obtained by the Kaplan-Meier method and log rank test in cervical cancer patients with locally advanced disease and age greater than 39 years, according to the recessive model for the *ERCC2* rs13181 polymorphism (nCC=16; nAA/AC=170)

Finally, through a combined analysis of both polymorphisms we observed that there is a statistically significant difference in overall survival between women with positive lymph nodes and different genotypes. So, it was possible verified that patients carriers of at least one C allele or T allele for *ERCC2* rs13181 and *XRCC1* rs1799782 polymorphisms, respectively, have a lower mean overall survival than the other women (p=0.034, Figure 18).

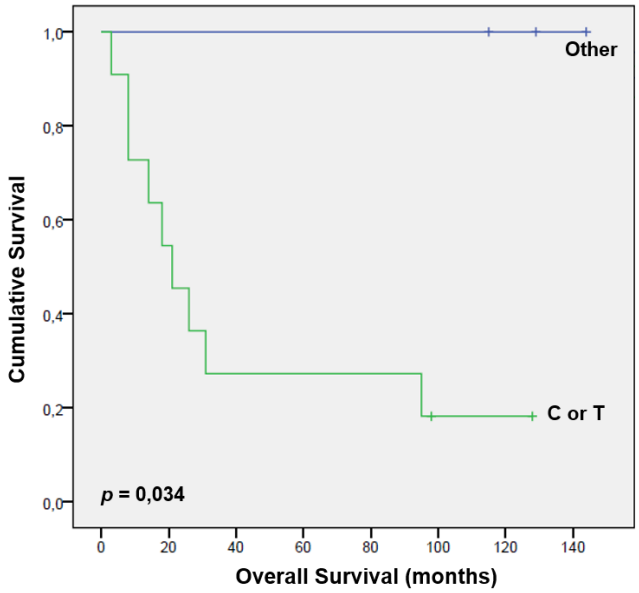


Figure 18 - Overall survival curves obtained by the Kaplan-Meier method and log rank test in cervical cancer patients with positive lymph nodes according to a combined analysis for both polymorphisms (CC/CA *ERCC2* or TT/CT *XRCC1* vs. AA *ERCC2* and CC *XRCC1*)

5. Association between disease-free survival (DFS) and *XRCC1* rs1799782 and *ERCC2* rs13181 polymorphisms

In analysis of the disease-free survival, the results showed that there were no statistically significant differences between different genotypes of the *XRCC1* rs1799782 polymorphism and this clinical outcome (p=0.816, Figure 19A). Regarding the *ERCC2* rs13181 polymorphism there was also no significant relationship between different genotypes and disease-free survival (p=0.473, Figure 19B). Similarly, applying the recessive model for polymorphism in the *ERCC2* gene (CC vs AA and AC) continue to exist no significant association with disease-free survival (p=0.228, Figure 20).

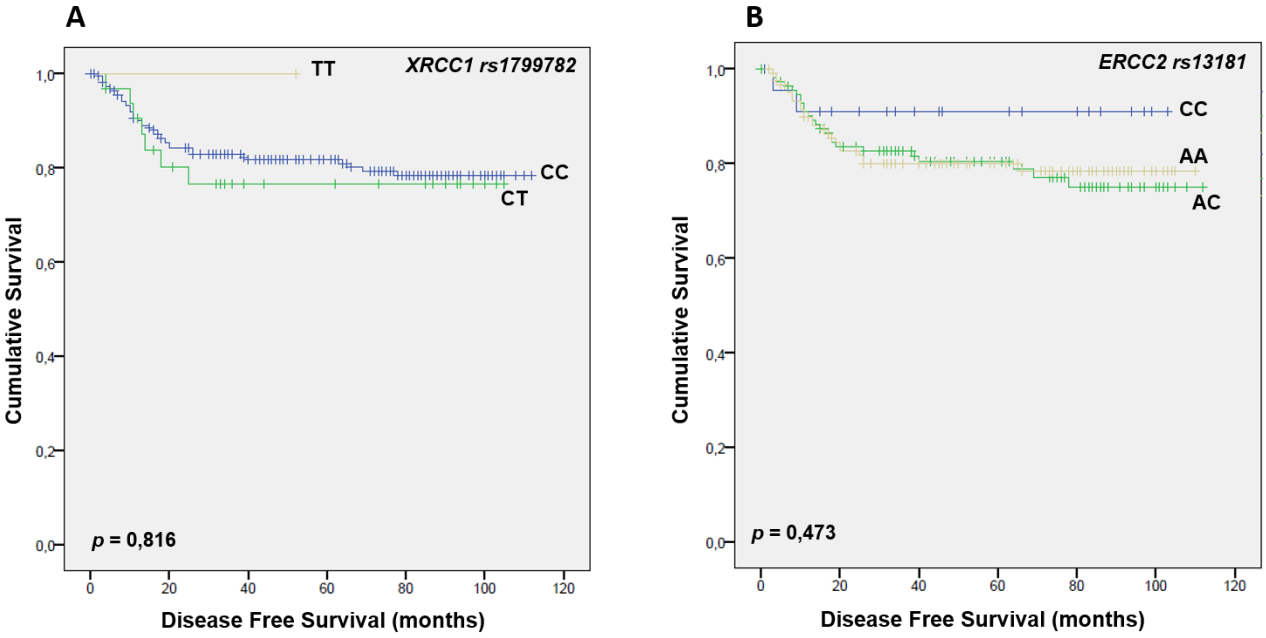


Figure 19 - Disease-free survival curves obtained by the Kaplan-Meier method and log rank test in cervical cancer patients, according to genotypes of the *XRCC1* rs1799182 (nTT=1; nCT=32; nCC=227) and *ERCC2* rs13181 (nCC=24; nAC=114; nAA=122) polymorphisms

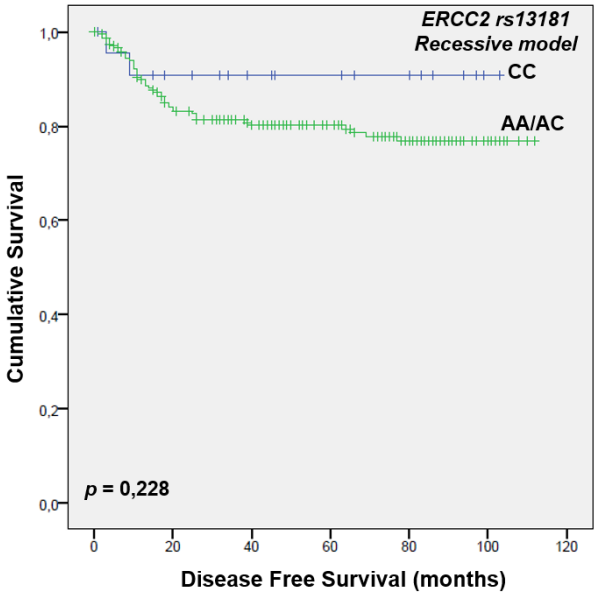


Figure 20 - Disease-free survival curves obtained by the Kaplan-Meier method and log rank test in cervical cancer patients, according to recessive model for *ERCC2* rs13181 polymorphism (nCC=24; nAC=114; nAA=122)

Similarly, to performed in overall survival, considering the recessive model for *ERCC2* rs13181 polymorphism, a statistical analysis was realized according to some clinical-pathological data, such as stage and age. The results showed that patients with *ERCC2* CC genotypes stage IIb or higher and age greater than 39 years present a statistically significant lower risk of developing relapse (*p*=0.040; Figure 21).

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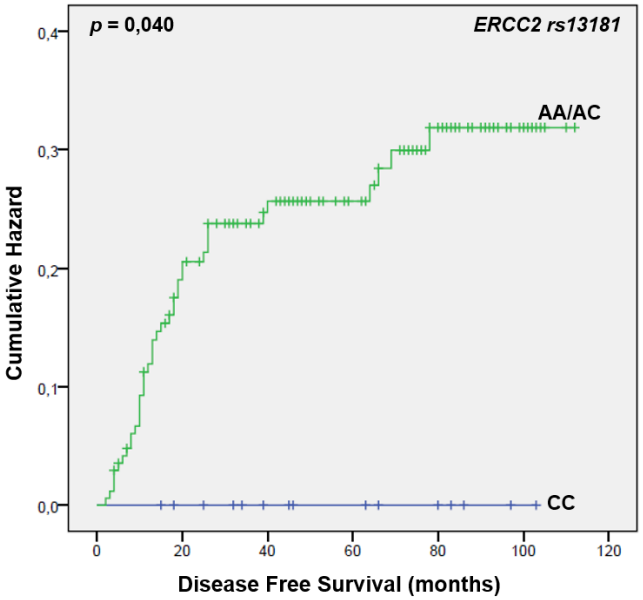
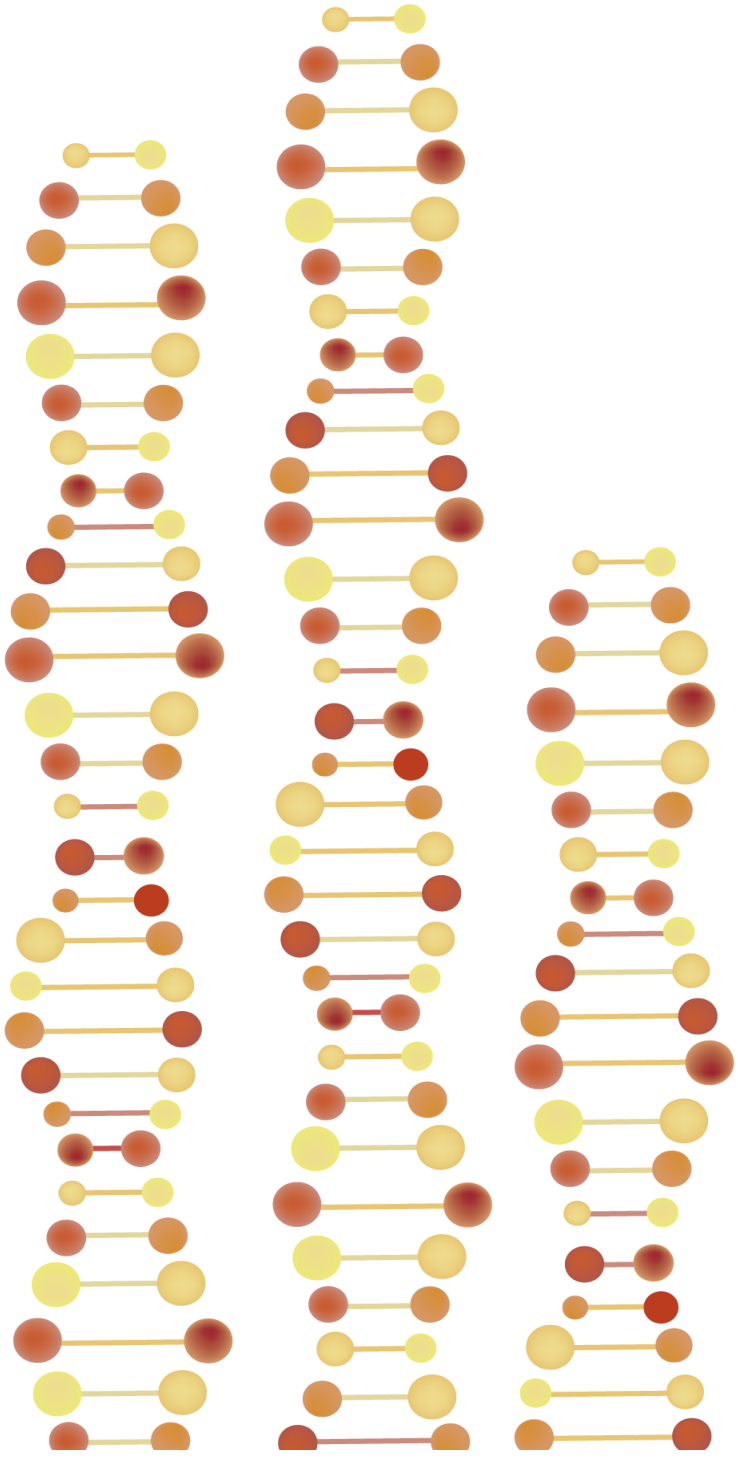


Figure 21 - Disease free survival curves obtained by the Kaplan-Meier method and log rank test in cervical cancer patients with a stage IIb or higher and age greater than 39 years according to the recessive model for the *ERCC2* rs13181 polymorphism (nCC=16; nAA/AC=172)

V. DISCUSSION



Despite rapid advances of investigation in molecular and cell biology areas, cellular mechanisms by which neoplastic cells progress and acquire their metastatic ability it is still widely studied. However, currently, it is known that carcinogenesis is a dynamic and non-linear process that depends on a large number of factors, regulated at multiple spatial and temporal scales, which makes cancer a highly heterogeneous disease. In turn, this heterogeneity has implied the need to establish new strategies for prevention, diagnosis and treatment for cancer [98].

Understand the role of individual genetic variability in cancer drugs efficacy and safety is a major challenge in current clinical practice, concerning to drug development and implementation for this disease. Thus, Pharmacogenomics has become a promising science, since it studies the impact of genetic variation on cancer drug efficacy and toxicity in order to reduce the trial-and-error approach in choice of treatment and, thereby, to limit the exposure of the patients to ineffective and toxic drugs [99, 100]. In this sense, genetic variation has been recognized as an important determinant factor of individual variability of drug response, since it may influence the dose-response curves and, consequently, the efficacy and toxicity drug in cancer patients [100]. Mutation events like insertions, deletions or chromossomal rearrangements can affect millions of base pairs, to leading variations in single nucleotides and results in DNA polymorphisms. Various types of polymorphism have been shown to responsible for variable and adverse cancer drug response, being that SNPs are the most promising for pharmacogenomic analysis [71]. When these genetic alterarions cause dysfunctions in genes encoding proteins involved in DNA repair pathways, can lead to increased genetic instability and, consequently, increased risk of cancer. In addition, can may confer resistance to cancer treatments and therefore can be considered as potential targets for oncologic therapy [51]. So, Pharmacogenomics predominantly is based on study of the SNPs, in order to fit individual genetic profile each patient, providing to an increase of safety and efficacy cancer treatment and, consequently, a longer patients survival and improvement of your life quality [71, 101].

In the present study, we analyzed two genetic polymorphisms, *XRCC1* rs1799782 and *ERCC2* rs13181, which are involved in distintic DNA repair pathways, BER and NER, respectively. Currently, despite the results remain controversial, there are several studies that have been demonstrating an association between these polymorphisms and development risk of different cancer types, including cervical cancer [59, 76, 80]. Relatively to the influence of these genetic variants in treatment response and clinical outcome of cervical cancer patients, few studies have been performed, so the existing results are considered preliminary and inconclusive [79, 83]. In this context, this study was developed with purpose of evaluating the effect of these polymorphisms on the

clinical evolution of Caucasian patients with diagnosis of cervical cancer treated with concomitant chemoradiotherapy. Thus, the response to treatment was evaluated, as well as the different clinical outcomes, namely overall survival and disease-free survival of patients, according to their clinicopathological characteristics.

1. Analysis of allele and genotype frequencies of the *XRCC1* rs1799782 and *ERCC2* rs13181 polymorphisms

In the present study, the allele frequencies of the *XRCC1* rs1799782 polymorphism were 93.5% and 6.5% for C and T alleles, respectively, being identical to whose reported in others European populations. When Hardy-Weinberg equilibrium was performed, no statistically significant difference was observed between observed and expected genotype frequencies (p=0.910). Regarding the *ERCC2* rs13181 polymorphism, the allele frequencies are similar to those observed in Europe, with a frequency of approximately 69% and 31% for A and C alleles, respectively. Concerning the distribution of genotypes, no statistically significant difference was verified between observed and expected frequencies, when applied Hardy-Weinberg equilibrium (p=0.721). The application of the Hardy-Weinberg equilibrium in studies that evaluate SNPs is very important, since that this assumption essentially states that unless there are counteracting forces, alleles frequencies will not change in a population and expected genotype frequencies each generation are determined by allele frequencies and are termed Hardy-Weinberg proportions (HWP) [102]. Deviation from these proportions can be caused by many factors, one of which is genotyping error [103].

In this study, we performed a comparison between allele and genotype frequencies obtained and those observed in other studies of different geographic areas and tumor models. The discrepancy in results between our population and Asian populations may be due to innate genetic diversity among ethnicities, as well as various interactive environmental factors such as climate, diet, and lifestyle. Factors related to the study design or sample size should also be put into consideration. On the other hand, much of the alteration in the allelic frequencies of certain populations may be due to the existence of migration, as well as to the gene flow of populations. The comparison of genotype frequencies between different populations becomes essential to show the importance of conducting additional studies including these polymorphisms, in order to corroborate the results obtained and to stablish possible associatons between these genetic variants and prognosis of cancer patients of different geographic regions.

In the present study, the results of genotyping were repeated in 10% of the cases, in order to increase the reliability and reproducibility of the results found. In addition, negative controls were also used in all amplification reactions, to ensure the absence of contamination of the genotyped samples. It is also to conclude that the quality and reliability of the Real-Time PCR technique reduces the possibility of being considered genotyping errors.

2. Association of the *XRCC1* rs1799782 polymorphism with response to chemoradiotherapy, overall survival and disease-free survival in patients with cervical cancer

It is well stablished that DNA damage repair is very important in the maintenance of genetic stability and protection against initiation of cancer development [79]. Genetic variations in genes involved in DNA repair could confer an increased tumoral susceptibility and can be associated to disease aggressiveness through of the alteration of DNA repair pathways, which can induce tumor transformation and acquisition of oncologic properties [85]. Currently chemoradiotherapy involving cisplatin is considered the standard treatment for some cancer types, including cervical cancer. Although cisplatin seems to be the most effective drug for treatment of cervical carcinoma, the cellular mechanisms dictating variable response to chemotherapy among patients are still unknown. The XRCC1 protein is a crucial member in BER pathway, and plays a pivotal role on repair DNA damage, including cisplatin-induced damage [87]. So, it was suggested that presence of SNPs in the *XRCC1* gene may alter the functional ability of *XRCC1* to repair damaged DNA, contibuting to variations in drug responses and, consequently, affect the success of treatment [79]. Regarding the risk of carcinogenesis, *XRCC1* rs1799782 polymorphism is well studied in both cervical cancer and other tumor models including head and neck, esophageal, gastric, breast, lung, colon cancers [59, 76, 80]. However, some studies have been evaluating the possible association between this polymorphism and chemotherapy response, but the results are inconsistente [79, 83]. Possibles explanations for this inconsistency of the results may be related with the different types of cancers studied from diverse ethnic populations and sample size.

Most studies that focus on assessing the possible influence of *XRCC1* rs1799782 polymorphism on clinical outcome of cancer patients undergoing cisplatin-based chemotherapy, include patients with non-small cell lung cancer (NSCLC) [87, 104, 105]. For example, Liu and colleagues performed a study including 322 NSCLC patients who received cisplatin-based chemotherapy and did not find significant association between *XRCC1* genetic variants and response to chemotherapy [87]. The same inconclusive

result was observed in the study of Zhao *et al*., with 147 NSCLC patients that received platinum-based chemotherapy [105]. On the other hand, Sun *et al*. identified an association between *XRCC1* genotypes and treatment response in 82 NSCLC patients. These authors verified that patients with CT genotype have a probability of about 2,33 times higher to develop a better response to treatment than CC genotype carriers [106]. In addition, a meta-analysis comprising 1145 lung cancer patients concluded also that patients carrying CT or TT genotypes were more likely to respond to platinum-based chemotherapy compared with CC genotype patients [104].

Others two studies including patients with pancreatic adenocarcinomas treated with surgery, radiation and chemotherapy (including platinum agents), showed that patients with CC genotype have a longer overall survival than patients with TT genotype [97, 107].

In cervical cancer, only two studies addressing the association between this polymorphism and chemotherapy response and different clinical outcomes [79, 83]. In first study, Kim *et al*. evaluated the existence of a possible correlation between polymorphisms in genes associated with DNA synthesis and repair and response to neoadjuvant platinum-based chemotherapy (NAC) and disease-free survival in patients with cervical cancer. This study included 66 patients and by combined analysis of the genotypes (CC vs. CT + TT), the results indicated that there is a significantly association between different *XRCC1* genotypes and response to NAC (p=0.023). Thus, patients with CC genotype presented poorer treatment responses than patients carrying CT and TT genotype, with CC genotype representative of the majority of poor responders. However, here was no correlation between *XRCC1* genotypes and DFS [83]. In second study were analysed 70 patients with locally advanced cervical carcinoma and the results obtained were opposite of the previous study. Cheng and colleagues did not find any significant association between *XRCC1* rs1799782 polymorphism and NAC response, justifying the inconsistency of the results with different ethnic populations studied and sample size [79].

In our study, in a general context the results showed that there was no statistically significant association between the genotypes of this polymorphism and therapeutic response (p=0.738), overall survival (OS) (p=0.761) and disease-free survival (DFS) (p=0.278). Thus, in relation to therapy response and disease-free survival is possible affirm that our results are consistent to those observed on studies of Cheng *et al*. and Kim *et al*., respectively, in which also no significant association was found between *XRCC1* genotypes and these clinical endpoints. However, in study of the Kim *et al*., was observed a correlation between this polymophism and treatment response, being this result contradictory to that obtained in our study. So, given that the studies published

concerning the effect of *XRCC1* rs1799782 polymorphism in prognosis of cervical cancer patients have been performed in Asian populations, will be needed additional studies in Caucasian populations in order to stablish more consistent conclusions. Moreover, there has been increasing evidence that decreased DNA repair capacity resulting from genetic polymorphisms of various DNA repair genes, namely *XRCC1* rs1799782, can be associated with improved survival of cancer patients treated with platinum-based chemotherapy. So, further studies would also be important to facilitate elucidation of the functional effect of this polymorphism in order to use it as a predictive and prognostic biomarker in cancer patients, particularly in patients with cervical cancer.

3. Association of the *ERCC2* rs13181 polymorphism with response to chemoradiotherapy, overall survival and disease-free survival in patients with cervical cancer

Resistance to platinum compounds takes place through of alterations in several cellular mechanisms, such as decreased drug accumulation, drug inactivation, enhanced tolerance to platinum-DNA adducts, or enhanced DNA repair. The NER pathway is one of the major DNA repair systems in mammalian cells and is principal activated repair mechanism for the removal of bulky DNA adducts produced by platinum agents. The ERCC2 gene, also known as *XPD*, encodes a helicase that is a component of transcription factor TFIIH and an essential member of the NER pathway, being that genetic alterations in this protein can result in defective DNA repair phenotypes [97]. Several polymorphisms in this gene have been the subject of intense investigation aimed at identifying functional consequences at the level cancer prognosis and susceptibility, being that *ERCC2* rs13181 polymorphism is the most studied. Regarding the risk of cervical carcinogenesis, the studies of Zhang *et al*. and Xiaohong *et al*. did not find any association between *ERCC2* genotypes and development risk of this neoplasia [97, 108]. Moreover, other studies have shown that this polymorphism plays a key role in tumorigenesis of different types of cancers, such as hepatocellular [109], lung [110], leukemia [111], melanoma [112], and pancreatic cancers [113]. Concerning the association of this polymorphis mand response to treatment, few studies have been conducted so far. In this sense, two studies have been conducted to evaluate whether *ERCC2* rs13181 polymorphism influence of the response to chemotherapy and survival in non-small lung cancer patients [96, 114]. In the study of Ryu *et al*., no statistically significant difference was found between the ERCC2 genetic variants both for response to therapy and overall survival [96]. In the other study, the authors found that the time of progression disease is significantly higher in patients with AC genotype than with AA

genotype patients [114]. Manoj *et al*. observed a significant increase in relapce-free survival but not in disease-specific survival in oral cancer patients with polymorphic genotype [93]. Park *et al*. conducted a study in patients with colorectal cancer and concluded that patients with AA genotype have a greater overall survival than patients with CC genotype [97].

Despite of various studies published, the functional effect of the ERCC2 rs13181 polymorphism has not yet been fully clarified, however it is thought that structural changes caused by the amino acid change may impact the interaction of *ERCC2* with other members of the NER complex leading to differential DNA repair ability. Furthermore, this polymorphism may cosegregate with other DNA repair enzymes such as *ERCC1* and *XRCC1* because of their close proximity in the genome, thus being a marker for DNA repair capacity without influencing *ERCC2* gene expression or protein function [115].

In order to contribute to the clarification of the *ERCC2* rs13181 polymorphism role in treatment efficacy and prognosis of cervical cancer patients, the present study appears to be the first to evaluate these possible associations. The results obtained did not indicate any statistically significant association between different *ERCC2* genotypes and therapeutic response (p=0.805), overall survival (p=0.279) and disease-free survival (p=0.473). Additionally, even by applying the recessive genetic model (CC vs. AA/AC), the results demonstrate that there is still no significant correlation for both overall survival (p=0.122) and dissease free-survival (p=0.228).

Given this, based on recessive model, a statistical analysis was performed according to the lymph nodes, which is an important prognostic factor to help guide treatment decisions in cervical cancer. So, it was found that patients with negative lymph nodes and carrying the CC genotype have a higher mean overall survival than women with at least one A allele (p=0.044). Additionally, patients were stratified according to other prognostic factor, namely disease stage, since is considered one of the most important clinical outcome factors in choice of treatment options in patients with diagnosis of cervical cancer. Thus, the stages stratification considered was stages inferior or equal to IIa versus stages superior or equal to IIb, since several authors consider this division as the most ideal between early stages and locally advanced or advanced stages [116]. The results showed that patients group with advanced disease and negative lymph nodes and who are carrying the CC genotype have a higher mean overall survival than women carriers of A allele (p=0.020).

The results obtained reinforce the importance of lymph nodes as a crucial prognostic factor of cervical cancer, influencing patient’s outcome and the choice of therapeutic

modality [117]. Despite the studied population presented advanced disease, the combination of negative lymph nodes and CC genotype results in higher overall survival. Biologically, this may be explained by the fact that the presence of C allele is apparently associated with decreased DNA repair capacity and, consequently, less viability of the tumor cells.

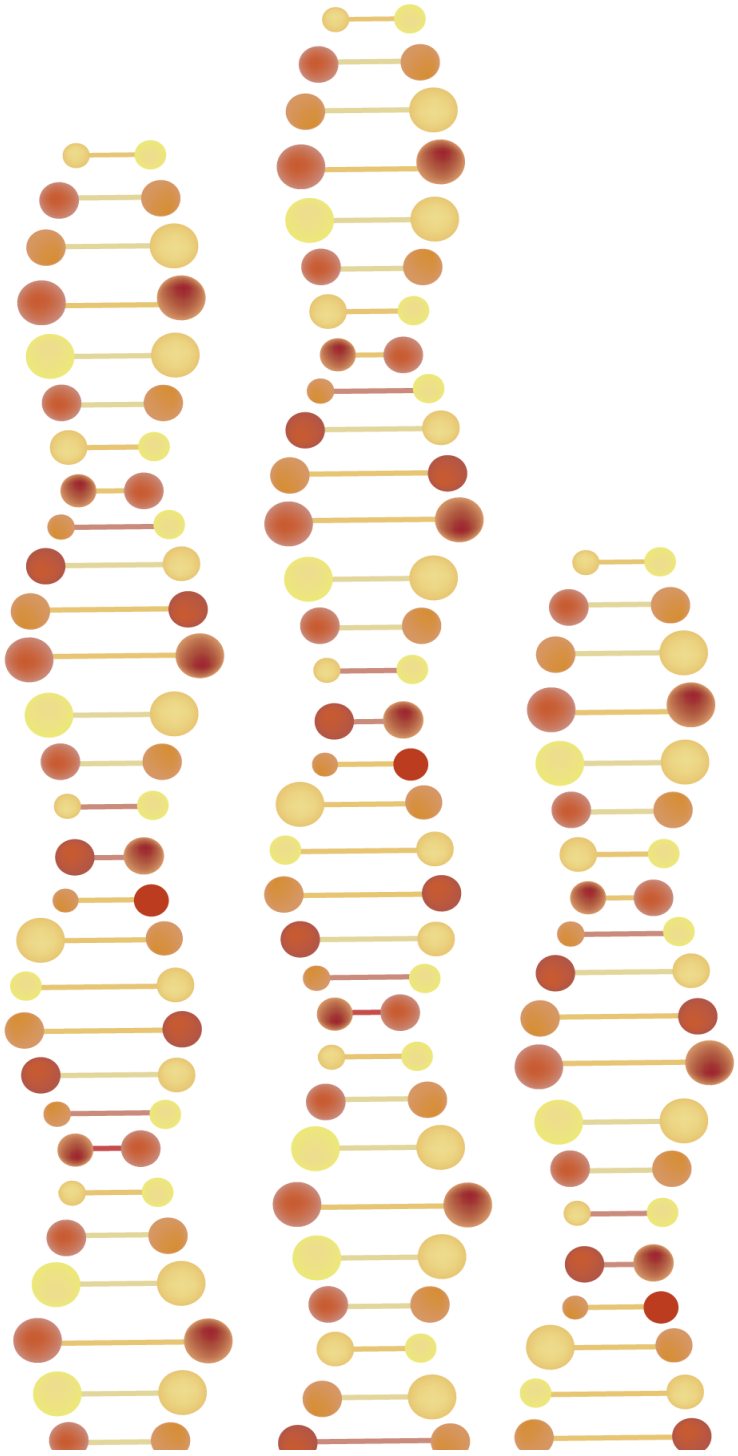
Furthermore, patients were also stratified by age, since is a well stablished prognostic factor for cervical cancer and seem be important in therapeutic decision making. In this sense, our results showed that women group with advanced disease and age greater than 39 years and present the CC genotype have a higher overall survival than women with at least one A allele (p=0.009). This result remains significant when performed a Cox regression analysis adjusted for prognostic factor lymph nodes, in which it was verified that patients with AA/AC genotypes present a risk of death of approximately 9 times higher than women with CC genotype (p=0.030). Additionally, in analysis corresponding to disease-free survival verified that patients with CC genotype and with advanced disease and age greater than 39 years present a statistically significant lower risk of developing relapse (p=0.040). Our results are consistent with others studies who suggest that younger age is an unfavorable prognostic factor, especially in more advanced stages [118, 119]. One possible explanation for this result is related with HPV virus infection, which is the main risk factor for cervical cancer development. HPV infection occurs as a large peak following sexual initiation which begins at approximately age 15-25 years and high-risk HPV types persist and progress to cancer within 10-15 years later. In addition, it is also at young age that women have multiple sexual partners, smoking cigarettes, immunocompromised hosts, a history of sexually transmitted diseases, and multiple high-risk HPV infections are therefore more likely to develop cervical cancer with aggressive characteristics. Thus, in older patients, the risk of developing high-risk HPV infections appears to be lower and, therefore, when the disease develops, it presents less aggressive tumor characteristics favoring a better prognosis [119].

Finally, a combined analysis of the two polymorphisms under study (*XRCC1* rs1799782 and *ERCC2* rs13181) was performed and results obtained indicated that patients with positive lymph nodes and carriers of at least one C or T allele for *ERCC2* and *XRCC1* polymorphisms, respectively, have a lower overall survival than the other patients (p=0.034). It can thus be concluded that in this case the polymorphisms biological effect is camouflaged by presence of positive lymph nodes since these seems to have more influence on the overall survival of patients with cervical cancer. That is, the presence of positive lymph nodes alone has a large negative effect on survival.

In order to understand whether *ERCC2* rs13181 polymorphism influence the repair capacity of platinum-induced damage, Zhang *et al*. developed an in vitro transfected cell model and obserevd that the alteration *ERCC2* AA to *ERCC2* CC causes a prolonged S-phase of cell cycle, leading to CC genotype cells presented a slight higher apoptosis rate. Thus, these authors found delayed recovery from S-phase cell cycle checkpoints in cells with CC genotype at the time point of 24 h after cisplatin treatment. In fact, the absence of the 'maintenance component' during S phase, contrary to the G1 and G2 checkpoints might be beneficial for cells by providing some delay but not permanent arrest with incompletely replicated genome. Long-term intra-S-phase blockade would limit the amount of sister chromatids and therefore reduce available DNA template for efficient repair [120]. Thus, by comparison of our results with Zhang *et al*. study we can hypothesize that the CC genotype is associated with a lower DNA repair capacity, resulting in presence of the damage levels increased. In addition, with the prolongation of the S-phase of the cell cycle and the late recovery of the checkpoints of this phase, which appears to exist in patients with CC genotype, damaged DNA is replicated, tagging the apoptosis activating pathways. Thus, there will be less viability of tumor cells with CC genotype and consequently a higher survival of these patients. Moreover, a negative lymph node status in patient's carriers CC genotype, which is a good prognostic factor, leads to a better response to therapy and consequently an increased overall survival.

Due to importance of the relationship between DNA repair and cancer therapy sucess, this research area is increasingly being explored. Thus, this study was conducted with the objective of contribute with new data regarding the influence of DNA repair genes polymorphisms on the clinical outcome of cancer patients, particularly in cervical cancer. In this sense, since the results obtained in our study are still preliminary, questions about the functionality of the *XRCC1* rs1799782 and *ERCC2* rs13181 polymorphisms should be addressed and their adjacent biological mechanisms need to be clarified. Therefore, there is a need to conduct further studies addressing these genetic variants with the outcome clinical of cervical cancer patients, in order to use these polymorphisms as valid predictive and prognostic biomarkers in this neoplasia.

VI. CONCLUSION AND FUTURE PERSPECTIVES



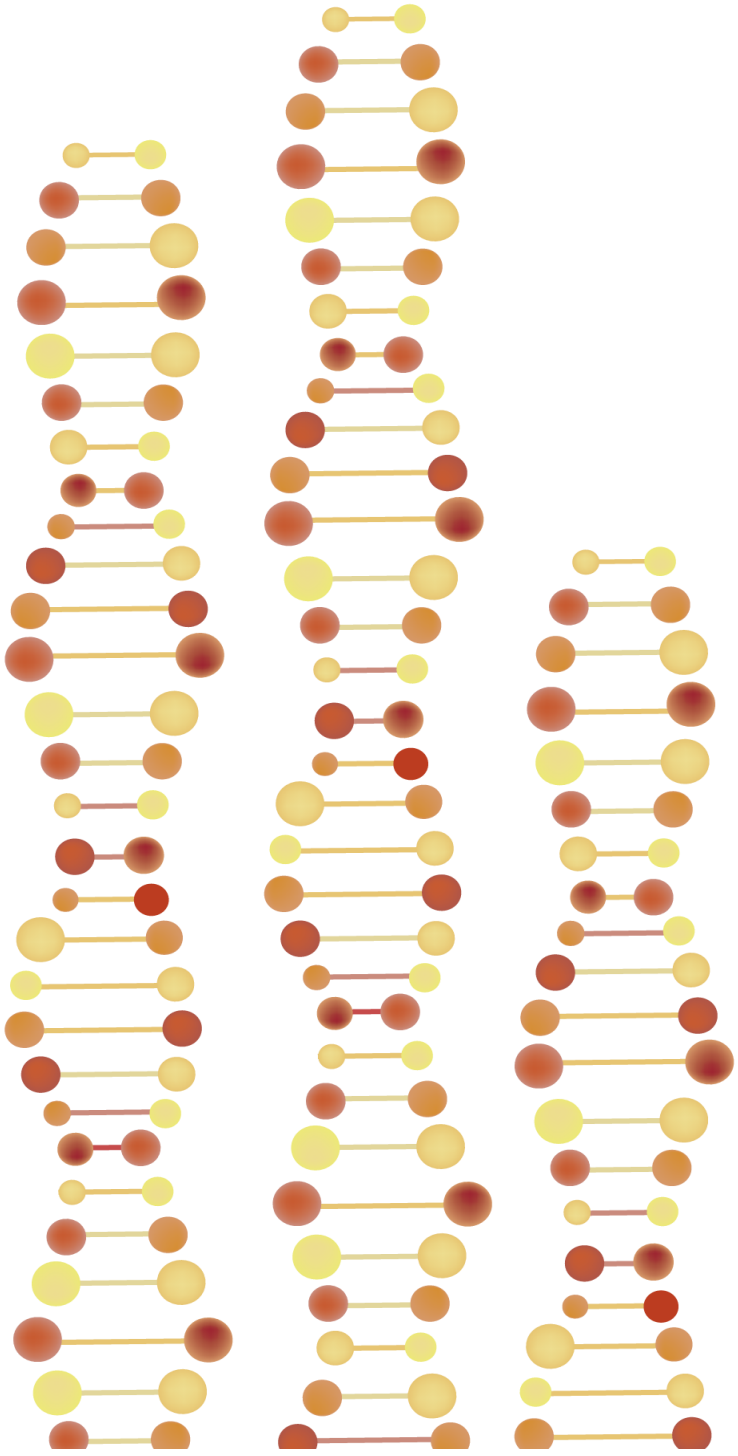
The role of genetic heterogeneity in neoplasias has become increasingly important in understanding the dynamics of cancer and resistance to therapy. Thus, the study of interindividual genetic variations, such as single nucleotide polymorphisms, has the potential to revolutionize medical practice by individualizing treatment, limiting patients' exposure to less effective or toxic drugs. Based on these genetic changes, the definition of susceptibility profiles for cancer development and predictors of patient's prognosis may be a useful tool in the implementation of prevention strategies and in the choice of treatment for each patient, in order to reduce incidence and mortality by cancer.

In this study, we highlighted the importance of polymorphisms in DNA repair genes as a potential biomarker for development and progression of cancer and as predictors of therapeutic response. In this context, two polymoprhisms were selected, namely *XRCC1* rs1799782 and *ERCC2* rs13181, in order to evaluate their influence in clinical outcome of patients with cervical cancer.

In a general way, the results showed that both polymorphisms analyzed do not influence the therapeutic response, overall survival and disease-free survival of patients. However, through analysis based on the recessive genetic model of *ERCC2* rs13181 polymorphism, it was found that women with CC genotype have an increased overall survival than carriers of A allele, taking into account some prognostic factors such as lymph nodes, stage and age. Moreover, it was also observed that patients with advanced disease, age over 39 years and with CC genotype have a lower risk of relapse than women with an A allele.

To summarize, it is important to highlight that the present study is the first to evaluate the role of *ERCC2* rs13181 polymorphisms as predictive and prognostic factor in patients with cervical cancer. Therefore, considering that the results obtained are still preliminary, it is important to carry out additional studies in cervical cancer, in order to validate the results and to futher clarify the putative functional effect of this polymorphism in clinical outcome of these patients. On the other hand, it would be interesting to study these polymorphisms in different ethinic populations in order to verify if these results can be extrapolated to worldwide population, independently of their ethnicity. Additionally, the evaluation of others polymorphisms in genes associated with DNA damage response, would be extremely important as it may help define a more individualized pharmacogenomic profile related with repair capacity of each patient.

VII. REFERENCES



1. Global Burden of Disease Cancer, C. et al. (2015) The Global Burden of Cancer 2013. JAMA Oncol 1 (4), 505-27.

2. Global Burden of Disease Cancer, C. et al. (2017) Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. JAMA Oncol 3 (4), 524-548.

3. RORENO (2015) Registo Oncológico Regional do Norte 2010. In: IPdOd, editor. Porto2015.

4. Weston A, Harris. CC. (2003) Multistage Carcinogenesis. 6th ed: BC Decker.

5. Kumat, V., A.K. Abbas, and J.C Aster. (2011) Robins Basic Pathology. 9th edition. Philadelphia: Elsevier Saunders.

6. Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: the next generation. Cell 144 (5), 646-74.

7. Ferlay, J. et al. (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136 (5), E359-86.

8. LaVigne, A.W. et al. (2017) Cervical cancer in low and middle income countries: Addressing barriers to radiotherapy delivery. Gynecol Oncol Rep 22, 16-20.

9. Ferlay, J. et al. (2013) Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer 49 (6), 1374-403.

10. Medeiros R, Ramada D. (2010) Knowledge differences between male and female university students about human papillomavirus (HPV) and cervical cancer: Implications for health strategies and vaccination. Vaccine, 29(2):153–60.

11. Ghebre, R.G. et al. (2017) Cervical cancer control in HIV-infected women: Past, present and future. Gynecol Oncol Rep 21, 101-108.

12. Dasari, S. et al. (2015) Cervical cancer: Biomarkers for diagnosis and treatment. Clin Chim Acta 445, 7-11.

13. Medeiros R, Prazeres H, Pinto D, Macedo-Pinto I, Lacerda M, Lopes C, et al. (2005) Characterization of HPV genotype profile in squamous cervical lesions in Portugal, a southern European population at high risk of cervical cancer. Eur J Cancer Prev., 14(5):467–71.

14. Silva, J. et al. (2011) Oncogenic HPV Types Infection in Adolescents and University Women from North Portugal: From Self-Sampling to Cancer Prevention. J Oncol 2011, 953469.

15. Ghittoni, R. et al. (2015) Role of human papillomaviruses in carcinogenesis. Ecancermedicalscience 9, 526.

16. Kessler, T.A. (2017) Cervical Cancer: Prevention and Early Detection. Semin Oncol Nurs 33 (2), 172-183.
17. Sawaya, G.F. and Huchko, M.J. (2017) Cervical Cancer Screening. Med Clin North Am 101 (4), 743-753.
18. Litwin, T.R. et al. (2017) Somatic Host Cell Alterations in HPV Carcinogenesis. Viruses 9 (8).
19. Marth, C. et al. (2017) Cervical cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 28 (suppl_4), iv72-iv83.
20. Patel, C.N. et al. (2011) 18F-FDG PET/CT of cervical carcinoma. AJR Am J Roentgenol 196 (5), 1225-33.
21. Heintz, A. et al. (2006) Carcinoma of the Ovary. Int J Gynaecol Obstet 95 Suppl 1, S161-S192.
22. Reinhardt, M.J. et al. (2001) Metastatic lymph nodes in patients with cervical cancer: detection with MR imaging and FDG PET. Radiology 218 (3), 776-82.
23. Choi, H.J. et al. (2006) Comparison of the accuracy of magnetic resonance imaging and positron emission tomography/computed tomography in the presurgical detection of lymph node metastases in patients with uterine cervical carcinoma: a prospective study. Cancer 106 (4), 914-22.
24. Koulis T.A., Kornaga E.N., Banerjee R, Phan T, Ghatage P, Magliocco AM, et al. (2017) Anemia, leukocytosis and thrombocytosis as prognostic factors in patients with cervical cancer treated with radical chemoradiotherapy: A retrospective cohort study., 4:51–6.
25. Teke F, Yoney A, Teke M, Inal A, Urakci Z, Eren B, et al. (2014) Lack of any impact of histopathology type on prognosis in patients with early-stage adenocarcinoma and squamous cell carcinoma of the uterine cervix. Asian Pacific J Cancer Prev., 15(6):2815–9. .
26. Intaraphet, S. et al. (2013) Prognostic impact of histology in patients with cervical squamous cell carcinoma, adenocarcinoma and small cell neuroendocrine carcinoma. Asian Pac J Cancer Prev 14 (9), 5355-60.
27. Manzo-Merino, J. et al. (2014) The role of signaling pathways in cervical cancer and molecular therapeutic targets. Arch Med Res 45 (7), 525-39.
28. Cossar, L.H. et al. (2017) Modulating the DNA Damage Response to Improve Treatment Response in Cervical Cancer. Clin Oncol (R Coll Radiol) 29 (9), 626-634.
29. Rogers, L. et al. (2009) Adjuvant radiotherapy and chemoradiation after surgery for cervical cancer. Cochrane Database Syst Rev (4), CD007583.
30. Klopp, A.H., Eifel P.J. (2011) Chemoradiotherapy for cervical cancer in 2010. Curr Oncol Rep 13 (1), 77-85.
31. Saxena, A. et al. (2005) Cellular response to chemotherapy and radiation in cervical cancer. Am J Obstet Gynecol 192 (5), 1399-403.

32. Haque, W. et al. (2017) Addition of chemotherapy to definitive radiotherapy for IB1 and IIA1 cervical cancer: Analysis of the National Cancer Data Base. Gynecol Oncol 144 (1), 28-33.

33. Ho, K.C. et al. (2011) Identification of prognostic factors in patients with cervical cancer and supraclavicular lymph node recurrence. Gynecol Oncol 123 (2), 253-6.

34. Katanyoo K, Sanguanrungsirikul S, Manusirivithaya S. (2012) Comparison of treatment outcomes between squamous cell carcinoma and adenocarcinoma in locally advanced cervical cancer. Gynecol Oncol, 125(2):292–6.

35. Stehman, F.B. et al. (2003) Innovations in the treatment of invasive cervical cancer. Cancer 98 (9 Suppl), 2052-63.

36. Pomeroy, M. and Moriarty, M. (1993) Clinical significance of cellular resistance in tumours to cytotoxic chemotherapy and radiotherapy. Cytotechnology 12 (1-3), 385-91.

37. Kurnia, I. Siregar, B. Soetopo, S. Ramli, I. Kurjana, T. Andriono, et al. (2014) Correlation Between Akt and p53 Protein Expression and Chemoradiotherapy Response in Cervical Cancer Patients. HAYATI J Biosci, 21(4):173–9.

38. Basu, A. Krishnamurthy, S. (2010) Cellular responses to Cisplatin-induced DNA damage. J Nucleic Acids 2010.

39. Dexheimer, T.S. (2013) DNA Repair of Cancer Stem Cells. Springer, 19-32.

40. Liu, Y. et al. (2016) Regulators in the DNA damage response. Arch Biochem Biophys 594, 18-25.

41. Mollaei, H. et al. (2017) The anti-proliferative and apoptotic effects of crocin on chemosensitive and chemoresistant cervical cancer cells. Biomed Pharmacother 94, 307-316.

42. Joiner MC, van der Kogel A. (2009) Basic Clinical Radiobiology. Fourth Edition: CRC Press.

43. Ho, C.K. et al. (2017) Expression of DNA damage response proteins in cervical cancer patients treated with radical chemoradiotherapy. Gynecol Oncol 145 (1), 176-184.

44. Goldstein, M. Kastan, M.B. (2015) The DNA damage response: implications for tumor responses to radiation and chemotherapy. Annu Rev Med 66, 129-43.

45. De Bont, R. van Larebeke, N. (2004) Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis 19 (3), 169-85.

46. Gurska, S. et al. (2007) Radiosensitivity of cervical cancer cell lines: the impact of polymorphisms in DNA repair genes. Neoplasma 54 (3), 195-201.

47. Close, D.M. Nelson, W.H. Bernhard, W.A. (2013) DNA Damage by the Direct Effect of Ionizing Radiation: Products Produced by Two Sequential One-Electron Oxidations. The Journal of Physical Chemistry.

48. Dasari, S. Tchounwou, P.B. (2014) Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol 740, 364-78.

49. Martin, L.P. et al. (2008) Platinum resistance: the role of DNA repair pathways. Clin Cancer Res 14 (5), 1291-5.
50. Goode, E.L. et al. (2002) Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev 11 (12), 1513-30.
51. Nowsheen, S. et al. (2012) Biomarkers to assess the targeting of DNA repair pathways to augment tumor response to therapy. Curr Mol Med 12 (6), 788-803.
52. Hosoya, N. Miyagawa, K. (2014) Targeting DNA damage response in cancer therapy. Cancer Sci 105 (4), 370-88.
53. Lord, C.J. et al. (2015) Synthetic lethality and cancer therapy: lessons learned from the development of PARP inhibitors. Annu Rev Med 66, 455-70.
54. Zharkov, D.O. (2008) Base excision DNA repair. Cell Mol Life Sci 65 (10), 1544-65.
55. Adhikari, S. et al. (2008) Targeting base excision repair for chemosensitization. Anticancer Agents Med Chem 8 (4), 351-7.
56. Izumi, T. Mellon, I. (2016) Base Excision Repair and Nucleotide Excision Repair. Genome Stability in Mammals in: Elsevier (Ed.) pp. 275-302.

57. Fortini, P. Dogliotti, E. (2007) Base damage and single-strand break repair: mechanisms and functional significance of short- and long-patch repair subpathways. DNA Repair (Amst) 6 (4), 398-409.

58. Hanssen-Bauer, A. et al. (2012) X-ray repair cross complementing protein 1 in base excision repair. Int J Mol Sci 13 (12), 17210-29.
59. Norjmaa B, Tulgaa K, Saitoh, T. (2016) Base Excision Repair Pathway and Polymorphisms of XRCC1 Gene. iMedPub Journals, 1-5.
60. Wilson, D.M. Akbari, M, Otterlei, M. (2013) The region of XRCC1 which harbours the three most common nonsynonymous polymorphic variants, is essential for the scaffolding function of XRCC1. DNA Repair (Amst), 11(7489):357–66.
61. London, R.E. (2015) The structural basis of XRCC1-mediated DNA repair. DNA Repair (Amst) 30, 90-103.
62. Shuai, H.L. et al. (2012) XRCC1 polymorphisms are associated with cervical cancer risk and response to chemotherapy: a systematic review and meta-analysis. Asian Pac J Cancer Prev 13 (12), 6423-7.
63. Gillet L.C, Scharer, O.D. (2006) Molecular mechanism of mammalian global genome nucleotide excision repair. Chem Rev., 106(2):253–76.
64. Benhamou, S. and Sarasin, A. (2002) ERCC2/XPD gene polymorphisms and cancer risk. Mutagenesis 17 (6), 463-9.
65. Hanawalt, P.C. (2002) Subpathways of nucleotide excision repair and their regulation. . Oncogene, 8949–56.

66. Costa, R.M. et al. (2003) The eukaryotic nucleotide excision repair pathway. Biochimie 85 (11), 1083-99.

67. Kinsella, T.J. (2009) Understanding DNA damage response and DNA repair pathways: applications to more targeted cancer therapeutics. Semin Oncol 36 (2 Suppl 1), S42-51.

68. Song, Y.Z. et al. (2015) ERCC2 polymorphisms and radiation-induced adverse effects on normal tissue: systematic review with meta-analysis and trial sequential analysis. Radiat Oncol 10, 247.

69. Merlo, L.M. and Maley, C.C. (2010) The role of genetic diversity in cancer. J Clin Invest 120 (2), 401-3.

70. Diaz-Padilla, I. Amir, E. Marsh, S. Liu, G. MacKay, H. (2012) Genetic polymorphisms as predictive and prognostic biomarkers in gynecological cancers: A systematic review. . Gynecol Oncol, 124(2):354–65.

71. Katara, P. (2014) Single nucleotide polymorphism and its dynamics for pharmacogenomics. Interdiscip Sci 6 (2), 85-92.

72. Børsting C, Morling, N. (2012) Single-Nucleotide Polymorphisms, Encyclopedia of Forensic Sciences, in: Elsevier (Ed.), pp. 233-238.

73. Regateiro, F. (2013) Genética médica, p. 355.

74. D'Errico, M. et al. (2017) Single nucleotide polymorphisms in DNA glycosylases: From function to disease. Free Radic Biol Med 107, 278-291.

75. Shen, W. Tian, Y. Ran, T. Gao, Z. (2015) Genotyping and quantification techniques for single-nucleotide polymorphisms. TrAC - Trends Anal Chem, 69:1–13.

76. Bajpai, D. et al. (2013) Decreased expression of DNA repair genes (XRCC1, ERCC1, ERCC2, and ERCC4) in squamous intraepithelial lesion and invasive squamous cell carcinoma of the cervix. Mol Cell Biochem 377 (1-2), 45-53.

77. Chaudhary, R. Singh, B. Kumar, M. Gakhar, S.K. Saini, A.K. Parmar, V.S. Chhillar, A.K. (2015) Role of single nucleotide polymorphisms in pharmacogenomics and their association with human diseases. Drug Metab Rev, 47(3):281–90.

78. Mei, J. et al. (2014) XRCC1 polymorphisms and cervical cancer risk: an updated meta-analysis. Tumour Biol 35 (2), 1221-31.

79. Cheng, X.D. et al. (2009) The association of XRCC1 gene single nucleotide polymorphisms with response to neoadjuvant chemotherapy in locally advanced cervical carcinoma. J Exp Clin Cancer Res 28, 91.

80. Yang, N.N. et al. (2017) Meta-analysis of XRCC1 polymorphism and risk of female reproductive system cancer. Oncotarget 8 (17), 28455-28462.

81. Liu, D.Y. Liang, H.C. Xiao, X.M. (2015) Association between the XRCC1 Arg399Gln polymorphism and risk of cervical carcinoma : a meta-analysis. Genetics and Molecular Research, 14(3):9821–8.

82. Ensemble (2018) rs1799782 SNP. http://www.ensembl.org/Homo_sapiens/Variation/Population, (accessed).

83. Kim, K. et al. (2008) XRCC1 Arginine194Tryptophan and GGH-401Cytosine/Thymine polymorphisms are associated with response to platinum-based neoadjuvant chemotherapy in cervical cancer. Gynecol Oncol 111 (3), 509-15.

84. Ensemble (2018) rs13181 SNP. http://www.ensembl.org/Homo_sapiens/Variation/Explore, (accessed).

85. Henríquez-Hernández, L.A. (2014) Single nucleotide polymorphisms in DNA repair genes as risk factors associated to prostate cancer progression. BMC Medical Genetics, 15:143.

86. Yan, Li, Q. Li, X. Ji, H. Zhang, L. (2016) Association studies between XRCC1, XRCC2, XRCC3 polymorphisms and differentiated thyroid carcinoma. Cell Physiol Biochem, 38(3):1075–84.

87. Liu, J.Y. et al. (2015) Association of GSTP1 and XRCC1 gene polymorphisms with clinical outcomes of patients with advanced non-small cell lung cancer. Genet Mol Res 14 (3), 10331-7.

88. Settheetham-Ishida, W. et al. (2011) Genetic risk of DNA repair gene polymorphisms (XRCC1 and XRCC3) for high risk human papillomavirus negative cervical cancer in Northeast Thailand. Asian Pac J Cancer Prev 12 (4), 963-6.

89. Hou, S.M. et al. (2002) The XPD variant alleles are associated with increased aromatic DNA adduct level and lung cancer risk. Carcinogenesis 23 (4), 599-603.

90. Hardi, H. Melki, R. Boughaleb, Z. Harroudi, T.E. Aissaoui, S. Boukhatem, N. (2018) Significant association between ERCC2 and MTHR polymorphisms and breast cancer susceptibility in Moroccan population : genotype and haplotype analysis in a case-control study. . BMC Cancer, 1-16.

91. Ni, M. et al. (2014) Association of ERCC1 and ERCC2 polymorphisms with colorectal cancer risk in a Chinese population. Sci Rep 4, 4112.

92. Shim, H.J. et al. (2010) BRCA1 and XRCC1 polymorphisms associated with survival in advanced gastric cancer treated with taxane and cisplatin. Cancer Sci 101 (5), 1247-54.

93. Mahimkar, M.B. et al. (2012) Polymorphisms in GSTM1 and XPD genes predict clinical outcome in advanced oral cancer patients treated with postoperative radiotherapy. Mol Carcinog 51 Suppl 1, E94-103.

94. Ravegnini, G. et al. (2016) Polymorphisms in DNA repair genes in gastrointestinal stromal tumours: susceptibility and correlation with tumour characteristics and clinical outcome. Tumour Biol 37 (10), 13413-13423.

95. Isla, D. Sarries, C. Rosell, R. Alonso, G. Domine, M. Taron, M. et al. (2004) Single nucleotide polymorphisms and outcome in docetaxel – cisplatin-treated advanced non-small-cell lung cancer. Annals of Oncology, 1194–203.

96. Ryu, J.S. Hong, Y.C. Han, H.S. Lee, J.E. Kim, S. Park ,Y.M. et al. (2004) Association between polymorphisms of ERCC1 and XPD and survival in non-small-cell lung cancer patients treated with cisplatin combination chemotherapy. Lung Cancer, 3–8.

97. Park, D.J. et al. (2001) A Xeroderma pigmentosum group D gene polymorphism predicts clinical outcome to platinum-based chemotherapy in patients with advanced colorectal cancer. Cancer Res 61 (24), 8654-8.

98. Grizzi, F. and Chiriva-Internati, M. (2006) Cancer: looking for simplicity and finding complexity. Cancer Cell Int 6, 4.

99. Lu, Q.M. (2011) Pharmacogenetics, Pharmacogenomics, and Individualized Medicine Pharmacological Reviews, 437-440.

100. Alwi, Z.B. (2005) The use of SNPs in pharmacogenomics studies. Malaysian Journal of Medical Sciences, 4-12.

101. Prunoiu, V.M. et al. (2015) The configuration of biomolecular markers in cancer of the uterine cervix. Personalized therapy. Monitoring and prognosis. Chirurgia (Bucur) 110 (2), 144-50.

102. Lancaster, A. et al. (2003) PyPop: a software framework for population genomics: analyzing large-scale multi-locus genotype data. Pac Symp Biocomput, 514-25.

103. Attia J, T.A., McElduff P, Milne E, Dawson S, Scott RJ, (2010) Detecting genotyping error using measures of degree of hardy-weinberg disequilibrium. Appl Genet Mol Biol, 9(1).

104. Cui, Z. Yin, Z. Li, X. Wu, W. Guan, P. Zhou, B. (2012) Association between polymorphisms in XRCC1 gene and clinical outcomes of patients with lung cancer: a meta-analysis. BMC Cancer, 12(1):71.

105. Zhao, W. et al. (2013) Polymorphisms in the base excision repair pathway modulate prognosis of platinum-based chemotherapy in advanced non-small cell lung cancer. Cancer Chemother Pharmacol 71 (5), 1287-95.

106. Sun, X. et al. (2009) Polymorphisms in XRCC1 and XPG and response to platinum-based chemotherapy in advanced non-small cell lung cancer patients. Lung Cancer 65 (2), 230-6.

107. Li, D. et al. (2006) Single nucleotide polymorphisms of RecQ1, RAD54L, and ATM genes are associated with reduced survival of pancreatic cancer. J Clin Oncol 24 (11), 1720-8.

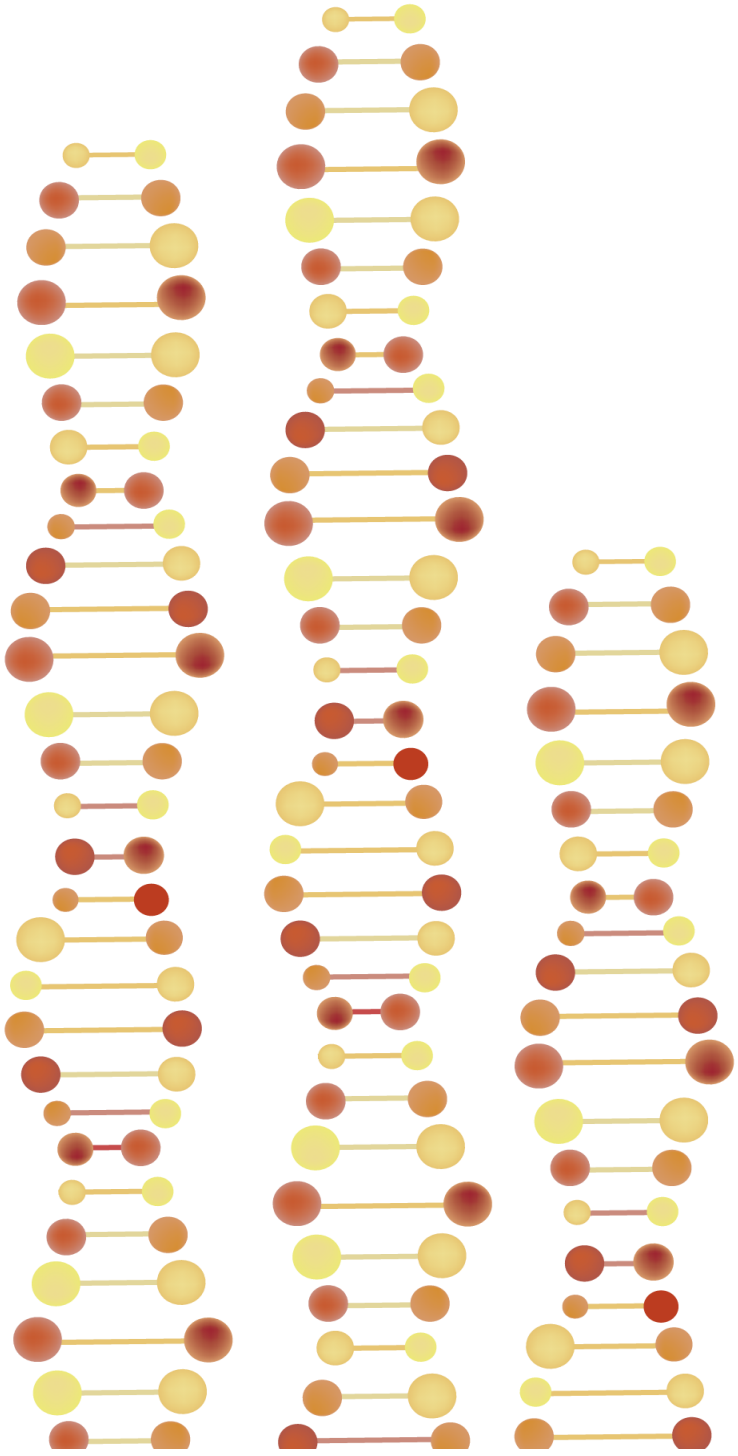
108. Zhang, L. et al. (2012) Single nucleotide polymorphisms in DNA repair genes and risk of cervical cancer: A case-control study. Oncol Lett 3 (2), 351-362.

109. Long, X.D. et al. (2009) XPD codon 312 and 751 polymorphisms, and AFB1 exposure, and hepatocellular carcinoma risk. BMC Cancer 9, 400.

110. Chang, J.S. et al. (2009) Base excision repair genes and risk of lung cancer among San Francisco Bay Area Latinos and African-Americans. Carcinogenesis 30 (1), 78-87.

111. Batar, B. et al. (2009) DNA repair gene XPD and XRCC1 polymorphisms and the risk of childhood acute lymphoblastic leukemia. Leuk Res 33 (6), 759-63.
112. Kertat, K. Rosdahl, I. Sun, X.F. Synnerstad, I. Zhang, H. (2008) The Gln/Gln genotype of XPD codon 751 as a genetic marker for melanoma risk and Lys/Gln as an important predictor for melanoma progression: A case control study in the Swedish population. Oncol Rep, 20(1):179–83.
113. He, M.G. et al. (2016) Association between ERCC1 and ERCC2 gene polymorphisms and susceptibility to pancreatic cancer. Genet Mol Res 15 (1).
114. Rosell, R. et al. (2003) Targeted therapy in combination with gemcitabine in non-small cell lung cancer. Semin Oncol 30 (4 Suppl 10), 19-25.
115. Dybdahl, M. Vogel, U. Frentz, G. Wallin, H. Nexø, B.A. (1999) Polymorphisms in the DNA repair gene XPD: Correlations with risk and age at onset of basal cell carcinoma. Cancer Epidemiol Biomarkers Prev., 8(1):77–81.
116. Duenas-Gonzalez, A. and Campbell, S. (2016) Global strategies for the treatment of early-stage and advanced cervical cancer. Curr Opin Obstet Gynecol 28 (1), 11-7.
117. Polterauer, S. et al. (2010) The impact of lymph node density on survival of cervical cancer patients. Br J Cancer 103 (5), 613-6.
118. Huang, H.J. et al. (2003) Prognostic value of age and histologic type in neoadjuvant chemotherapy plus radical surgery for bulky (>/=4 cm) stage IB and IIA cervical carcinoma. Int J Gynecol Cancer 13 (2), 204-11.
119. Nartthanarung, A. et al. (2014) Age and survival of cervical cancer patients with bone metastasis. Asian Pac J Cancer Prev 15 (19), 8401-4.
120. Bartek, J. and Lukas, J. (2001) Mammalian G1- and S-phase checkpoints in response to DNA damage. Curr Opin Cell Biol 13 (6), 738-47.

VIII. APPENDIXES



Appendix I

Abstract accepted for poster presentation in Eurogin congress, Lisbon, 2018.

XRCC1 rs1799782 and *ERCC2* rs13181 POLYMORPHISMS AS POTENTIAL PROGNOSTIC AND PREDICTIVE FACTORS IN CERVICAL CANCER PATIENTS

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Background/Objectives: Cancer cells efficiently repair treatment-induced DNA damage, exhibiting greater resistance to radiation or DNA damaging agents. Cervical cancer is commonly treated by platinum-based chemoradiotherapy, and the inactivation of DNA repair may increase the efficacy of treatments. Given that genetic polymorphisms seem to influence the repair capacity of tumor cells and can be identified by using blood samples, they are promising biomarkers in the clinical decision-making process for cancer patients. Thus, the aim of present study was to assess the prognostic and predictive values of *XRCC1* rs1799782 and *ERCC2* rs13181 polymorphisms in cervical cancer patients.

Material/Methods: This retrospective hospital-based study includes a total of 260 Caucasians patients with histologically confirmed cervical carcinoma (FIGO stage IB2-IVA). The patients were recruited between February 2002 and October 2009 and treated with cisplatin-based concomitant chemoradiotherapy in Portuguese Institute of Oncology of Porto. The genotyping was performed using Taqman™ Allelic Discrimination methodology by Real-Time PCR. Difference in frequencies of the genotype between the different therapy responses groups were evaluated by χ^2 test. Overall survival (OS) and disease-free survival

(DFS) were estimated by Kaplan-Meier method and log-rank test. A level of P<0.05 was considered statistically significant.

Results: There were no significant statistical differences between the different genotypes of the *XRCC1* rs1799782 and *ERCC2* rs13181 polymorphisms and treatment response (P=0.738 and P=0.805, respectively). Concerning the OS, we observed that patients with advanced disease, negative lymph nodes metastasis (LNM) and carriers of *ERCC2* CC genotypes present a higher survival when compared with carriers at least one A allele (AA/AC genotypes) (P=0.020). Additionally, we verified that carriers *ERCC2* CA/AA genotypes carrier patients present a risk of death of approximately 9 times higher than patients with the CC genotype, adjusted for LNM prognostic factor (*P*=0.030; *P*=0.029, bootstrap analysis).
The results also showed that patients group with stage IIb or higher, age above 39 years old and carriers of *ERCC2* CC genotypes present a statistically significant lower risk of developing relapse than CA/AA genotypes carrier patients (*P*=0.040).

Conclusions: In conclusion, we demonstrated the clinical significance of polymorphisms in DNA repair genes in cervical cancer patients. The *ERCC2* rs13181 polymorphism might be used as a prognostic marker for patients undergoing cisplatin-based chemoradiotherapy. However, additional studies are required for validation these results.

Appendix II

Scientific article submitted for publication in *Biomarkers* journal entitled:

THE INFLUENCE OF *XRCC1* RS1799782 AND *ERCC2* RS13181 GENETIC POLYMORPHISMS IN TREATMENT RESPONSE AND PROGNOSIS IN CERVICAL CANCER PATIENTS.

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